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THE CHEMISTRY OF NATURAL COLORING MATTERS

The Constitutions, Properties, and Biological Relations of the Important Natural Pigments

BY

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in the University of Frankfort-on-Main

TRANSLATED AND REVISED
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GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the society, Washington, D. C.; the late John E. Teeple, then treasurer of the society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A. C. S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed editors (the present list of whom appears at the close of this introduction) to have charge of securing authors, and of considering critically the manuscripts submitted. The editors endeavor to select topics of current interest, and authors recognized as authorities in their respective fields.

The development of knowledge in all branches of science, especially in chemistry, has been so rapid during the last fifty years, and the fields covered by this development so varied that it is difficult for any individual to keep in touch with progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and by such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie, Moissan's Traité de Chimie Minérale Générale, Friend's and Mellor's Textbooks of Inorganic Chemistry and Heilbron's Dictionary of Organic Compounds, it often takes a great deal of time to coördinate the knowledge on a given topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value. It was with a clear recognition of the usefulness of such work that the American Chemical Society undertook to sponsor the publication of the two series of monographs.

Two distinct purposes are served by these monographs: the first, whose fulfillment probably renders to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a form intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs enable such men to form closer contact with work in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made, and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, extended references to the literature enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection is made of those papers which are most important.

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Introduction

The present volume represents an attempt to survey in the English language the chemical relations known to exist among natural compounds which possess visible color.

Until the middle of the last century coloring matters of natural origin were the only ones used in technical practice. The task of unravelling the constitution of such substances,[1] *e.g.*, alizarin and indigo, was intimately connected with and in part responsible for the remarkable expansion of organic chemistry at that time; there followed however a period when interest in the natural coloring matters was relatively weakened by the triumph of synthetic dyestuffs. Research in the field of natural coloring substances never completely ceased, but it regained considerable momentum only in the last two decades, and then to such effect that it is true to say that the progress made in this time completely overshadows that of the whole preceding period. It was fortunate also that the conjunction of biochemical and purely chemical paths at this same time first revealed the biological significance of many natural pigments, and this knowledge in turn stimulated still further their chemical examination.

Admittedly our present knowledge is so inadequate in many directions that a large number of investigations mentioned in this volume have no known biological implications, and the mere appearance of color in a natural material has been sufficient incentive to study the compound responsible. Whatever the motive for the study may have been, today the number of pigments the constitution of which still presents an unsolved problem is probably quite small and is constantly becoming smaller. For this satisfactory state Pregl's methods of microanalysis which have facilitated work in so many fields are in large measure responsible. Besides this achievement of the pure organic chemist, it is regrettable that even today we remain almost completely ignorant of the methods by which these pigments are elaborated by nature and in only few instances has the veil been even partially lifted.[2] These then are some of the considerations which seem to make the present time an opportune one for the presentation of this account.

The author of an account of synthetic dyestuffs must constantly bear in mind the two desirable properties which entitle a compound to be included in this group, *i.e*, color and affinity [3] for the fiber. This volume, on the other hand, considers the most important of those compounds which play some rôle, too often unknown, as pigments in nature, and their inclusion has no reference to their substantivity.

---

[1] *See* Cady: *Am. Dyestuff Reptr.*, 26, 539 (1937).

[2] *See* Schöpf: *Ann.*, 497, 1 (1932).

[3] *See* Fritz Mayer, "Chemie der organischen Farbstoffe," Vol. 1, p. 1, Berlin. 1935.

The arrangement follows the usual conventions of organic chemistry to preserve the character of a chemical textbook, but no claim is made to have included all known pigments or all the known facts relating to the compounds which do find a place here. The selection of material has been made with reference to two broad guiding principles. First an attempt has been made to provide a work to which reference may be made for the constitutions and significant chemical and physical properties of the important natural pigments. Secondly, it is hoped that the brief descriptions of the arguments for assigning the constitutions given have resulted in a picture of one phase of chemical thought of the past and present, and that this picture may be of some assistance to the student and research worker. Finally, although biological relations are mentioned only in passing, some mention of them was felt to be desirable, as they probably constitute the outlines of the picture of organic chemical thought of the future.

Foreword

The third German edition of the second volume of the author's "Chemie der organischen Farbstoffe," containing an account of natural coloring matters, appeared in 1935. The intervening years have witnessed striking progress in almost all branches of the subject; in spite of the short lapse of time, therefore, the German edition was felt to be in many respects misleading, if not obsolete, and the present volume is a completely revised text based on the former German work. The opportunity has been taken to include an account of some natural pigments which formerly escaped notice; but only coloring matters which have been characterized by at least analytical figures and significant chemical properties are mentioned.

The literature has been revised from *Chemisches Zentralblatt* up to the issue of October 1, 1939.

My sincerest thanks are due to Dr. A. H. Cook for his translation which successfully maintains the character of my German study, and I thank him once more for his careful consideration of the subject matter of the manuscript and for many features for its improvement.

Suggestions from readers regarding further improvements or elimination of errors will be heartily welcomed.

FRITZ MAYER.

London, England,
November 1, 1939.

Translator's Note

It was the translator's privilege to have spent many happy hours discussing almost every detail of the following text in harmonious collaboration with Professor Mayer. Both eagerly anticipated the joint task of proof reading. It was therefore a profound shock to hear that Professor Mayer died in July, 1940, in circumstances which, it need only be said, were as sad as any imaginable. The translator would like to pay tribute to a chemist whose erudition was evident to the whole world through his writings and whose human qualities, among them a particularly charming Old-World courtesy, must stimulate the happiest recollections in all privileged to have known him.

Meanwhile war-time conditions of transport, etc., have caused more delay than was anticipated between completing the manuscript and proof reading. Opportunity has been taken therefore to revise the text to include recent findings up to the summer of 1941. Where technical difficulties have rendered impossible inclusion of the most recent advances in the text, these have found some mention in footnotes, and so it is hoped that no significant number of contributions has been overlooked.

A. H. COOK.

London, England,
August, 1941.

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Chapter 1

Carotenoids (Polyene Pigments)[1]

Among the pigments of known, or at least partially known, biological significance which the mammalian organism obtains by way of its food, are the lyochromes and the lipochromes;[5] they represent, however, sharply divergent chemical types.

The term *lipochrome* indicates occurrence in association with fatty substances and the name *lyochrome* refers to their solubility in water. Older names for the carotenoids are: luteins, chromolipoids and lipoxanthins. The name *polyene pigments* indicates their relation to the polyene series ("ene" signifying a double linkage, according to the Geneva nomenclature). The table on page 12 compares the outstanding properties of the lipochromes and lyochromes.[3]

Botanists and chemists since the time of Berzelius have investigated the carotenoids and amassed a vast amount of information on their occurrence, recognition and physical properties. Willstätter[4] and his collaborators were the first to succeed in isolating pure compounds from plant material and in establishing their composition, although Willstätter expressed doubt of the homogeneity of some of his preparations, a doubt which was later justified. Nevertheless the methods elaborated by him form the basis of present research. A second important step forward was made when Zechmeister[5] demonstrated by catalytic hydrogenation that carotene contains an essentially aliphatic structure, Karrer[6] arriving almost simultaneously at a similar conclusion with respect to crocetin.

---

[1] General literature: Kohl, F. G.: "Untersuchungen über das Carotin und seine physiologische Bedeutung in der Pflanze," Leipzig, 1902. Tswett, M.: "Chromophylls in Plant and Animal Kingdoms," Warsaw, 1910 (Russ). Willstätter, R., and Stoll, A.: "Untersuchungen über Chlorophyll, Methoden und Ergebnisse," Berlin, 1913; "Untersuchungen über die Assimilation der Kohlensäure," Berlin, 1918. Palmer, L. S.: "Carotinoids and Related Pigments," New York, Chemical Catalog Co., Inc. (Reinhold Publishing Corp.), 1922. Lubimenko, V. N., and Brilliant, V. A.: "Colour of Plants," Leningrad, 1924 (Russ.). Mayer, F., "Carotinoide," in Meyer, V., and Jacobson, P.: "Lehrbuch der organischen Chemie," **II,** 5, 1, p. 164, Berlin and Leipzig, 1929. Zechmeister, L.: "Carotinoide höherer Pflanzen in Klein, Handbuch der Pflanzenanalyse," Berlin, 1932. Lederer, E.: "Les Caroténoides des Plantes," Paris, 1934. Zechmeister, L.: "Carotinoide," Berlin, 1934. Lederer, E.: "Les Caroténoides des Animaux," Paris, 1936. Gilman, H., "Organic Chemistry," Vol. 2, p. 1139, New York, 1938. Zechmeister, *Erg. Physiologie,* **30,** 117 (1937). Constitution and physiological significance of carotenoids: Morton, *Chem. and Ind.,* **59,** 301 (1940). *See also* Beilstein, "Handbuch der organischen Chemie," **30** (1938).

[2] The term "Carotenoid" was proposed by Tswett in 1911, *cf.* also Kuhn, Grundmann: *Ber.,* **65,** 1880 (1932); Vogel, Stohl: *Ber.,* **66,** 1066 (1933).

[3] Kuhn, György, Wagner-Jauregg: *Ber.,* **66,** 1034 (1933).

[4] Willstätter, Mieg: *Ann.,* **355,** 1 (1907); *cf.* also Zechmeister: "Die Forschungen Richard Willstätters auf dem Gebiete der Carotinoide," *Naturwiss.,* 20, 608 (1932).

[5] Zechmeister, v. Cholnoky, Vrabély: *Ber.,* **61,** 566 (1928); cf. Liebermann, Mühle: *Ber.,* **48,** 1653 (1915); Herzig, Faltis: *Monatsh.,* **35,** 997 (1914).

[6] Karrer, Salomon: *Helv. Chim. Acta,* **11,** 513, 711 (1928).

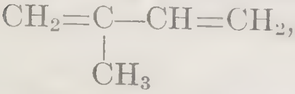
Composition	Lyochromes Nitrogeneous	Lipochromes Non-nitrogenous
Solubility in water	Soluble	Insoluble *
Color of solution	Yellow-orange	Yellow-red
Fluorescence	Strong green	Weak yellow-green †
Prosthetic group bound to	Protein	Protein in the pigments of lobster and other Crustaceae ‡
Behavior toward:		
acids	Stable	Very sensitive
alkalies	Sensitive	Stable
oxidizing agents	Very stable	Very sensitive
Biological relation	Vitamin B_2 and oxidation ferments	Vitamin A
Effective dose rat/day	5γ Lactoflavin	5γ, α- or γ-carotene 2.5γ β-carotene

* Crocin, occurring in saffron, in which the water-insoluble crocetin is bound glucosidically forms an exception.

† In spite of statements to the contrary in the literature, carotene and lycopene fluoresce, though only weakly.

‡ That carotenoids occur in association with proteins in Crustaceae is well known; spectral evidence goes to show that this type of association is quite general though its destruction is not always accompanied by striking changes in color. Cf. Menke, *Naturwiss.*, **28**, 31 (1940).

Meanwhile, however, the preparation by Kuhn[7] of synthetic polyene compounds containing aromatic residues at both ends of the chain and their similarity to carotene, their light absorption and their behavior toward perbenzoic acid and iodine chloride[8] provided convincing evidence of the polyene nature of the carotenoid pigments. During the last ten years the chemistry of the carotenoids has been actively investigated and in many respects clarified, particularly by Karrer, Kuhn and Zechmeister.

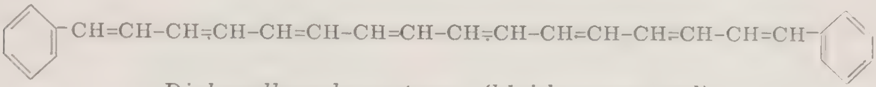
The differences between the synthetic and the natural polyenes are not very marked. Some of these diphenylpolyenes behave on degradation in a manner recalling the natural carotenoids. Diphenyloctatetrene, for example, is oxidized by acid permanganate to phenylheptatrienal. It is curious, in view of previous statements of the inability of such polyenes to exist in more than one steric form, that the resulting aldehyde is not identical with one synthesized earlier.[9] In nature the unit appearing so often in hydrocarbons and their derivatives is isoprene:  $CH_2{=}\underset{\underset{CH_3}{|}}{C}{-}CH{=}CH_2$, so that in the natural polyenes there occur methyl groups usually in the 1:5 position instead of a simple chain of methine groups. Again, whereas the synthetic polyenes of Kuhn carry phenyl residues at both ends of the chain, the natural polymethine chains are stabilized by terminal methyl or carboxyl groups, or by terpene rings which may be regarded as derived from isoprene. The following formulas illustrate the similarity:

---

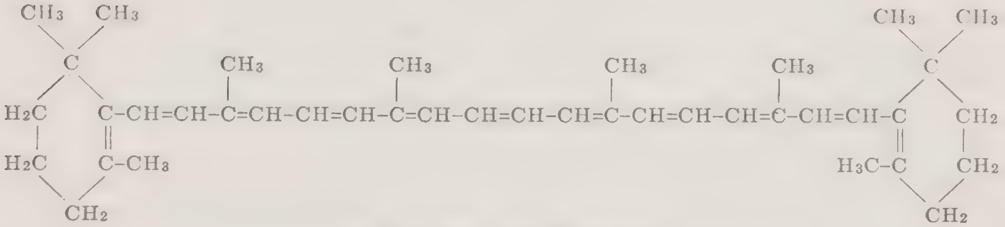
[7] Kuhn, Winterstein: *Ibid.*, **11**, 87, 116, 123, 144 (1928). Kuhn: *Angew. Chem.*, **50**, 703 (1937) (review).

[8] Pummerer, Rebmann: *Ber.*, **61**, 1099 (1928).

[9] Karrer and Obst, *Helv. Chim. Acta*, **22**, 1191 (1939).

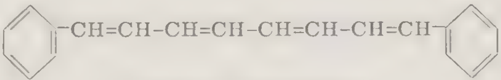


Diphenylhexadecaoctaene (bluish-copper red)



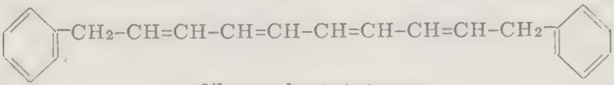
β-Carotene (dark violet)

Thus the color of the carotenoids is due to a long chain of conjugated double bonds. The conjugated nature of the double-bonded system in carotene is confirmed by the preparation of an addition product containing 5 molecules of maleic anhydride.[9a] The instability of compounds with a plurality of unsaturated residues, in both synthetic and natural polyenes, is counteracted by the presence of stabilizing groups. The effect of increasing conjugation is seen on comparing



diphenyloctatetrene[10]

which is greenish yellow in color with



dibenzyloctatetrene

which is colorless. Again, conversion of *bixin* into dihydrobixin (bixin carries terminal carboxyl groups) results in a lightening in color from red to yellow.[11]

Measured by the effect on the color, a carboxyl or phenyl group conjugated with a polyene chain is equivalent[12] to approximately 1½ double linkings. It will be noticed that, in the formula for β-carotene shown above, the two central methyl groups are not in the 1:5 but in the 1:6 position relative to each other, and it is accordingly convenient to assume that two isoprene nuclei have been linked as in (b) rather than as in (a) so that the pigment assumes a symmetrical structure.

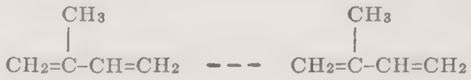
[9a] Nakamiya, *Bull. Inst. Phys. Chem. Res., Tokyo*, 15, 286 (1936).

[10] Kuhn, Winterstein: *Helv. Chim. Acta*, 11, 123 (1928).

[11] Karrer, Helfenstein, Widmer, van Itallie: *Ibid.*, 12, 741 (1929).

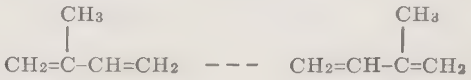
[12] Kuhn, Winterstein: *Ibid.*, 12, 899 (1929).

(*a*)



$$CH_2{=}\overset{\overset{CH_3}{|}}{C}{-}CH{=}CH_2 \quad --- \quad CH_2{=}\overset{\overset{CH_3}{|}}{C}{-}CH{=}CH_2$$

(*b*)



$$CH_2{=}\overset{\overset{CH_3}{|}}{C}{-}CH{=}CH_2 \quad --- \quad CH_2{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH_2$$

The composition of plant products which may be regarded as derived from isoprene indicate three processes of formation:

(1) By direct addition of C_5H_8 residues one to another, yielding terpenes; in this way the formation of the whole derived group, as well as of the hydrocarbon residues, may be explained.

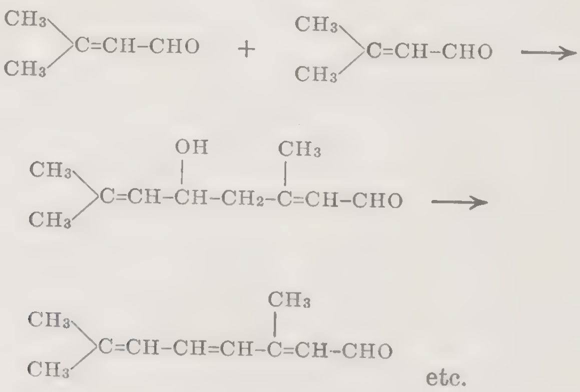
(2) By addition and simultaneous reduction,[13] as is to be assumed in the formation of phytol (see Chlorophyll):

$$4C_5H_8 + H_2O + 3H_2 = C_{20}H_{40}O$$

(3) By addition and dehydrogenation, so that polyenes with conjugated double bonds are formed:[14]

$$8C_5H_8 - 4H_2 = C_{40}H_{56}$$

The formation of carotenoids containing 40 carbon atoms might conceivably proceed through phytol, but experiment tends to show that the quantity[15] of phytol present in the plant is insufficient to account for the carotenoid content. The possibility that β-methylcrotonaldehyde[16] functions as a synthetic unit has also been considered:



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[13] Willstätter, Mayer, Hüni: *Ann.*, **378**, 73 (1911).
[14] Kuhn, Winterstein: *Helv. Chim. Acta*, **11**, 427 (1928).
[15] Kuhn, Brockmann: *Z. physiol. Chem.*, **206**, 41 (1932); Kuhn, Grundmann: *Ber.*, **65**, 1886 (1932).
[16] Karrer, Helfenstein, Widmer: *Helv. Chim. Acta*, **11**, 1201 (1928).

Up to the present, carotenoids found in nature may be subdivided into:

(1) Hydrocarbons
(2) Oxygenated compounds containing
    a. hydroxyl, aldehyde, and ketone groups
    b. carboxyl groups.

Kuhn and Winterstein [17] have postulated that a carotenoid containing 40 atoms of carbon is the precursor of natural polyene carboxylic acids of low molecular weight, so that formation of the polyene acids is a secondary reaction.

In connection with the form in which carotenoids are found in a plant, it may be mentioned that those pigments containing hydroxyl groups are frequently encountered as esters of fatty acids; such esters are termed *Farbwachse* [18] on account of their low melting point and the consistency of the crystals. *Physalien,* the pigment of the winter-cherry (*Physalis*), the dipalmitic ester [18] of *zeaxanthin,* was the first of this group to be studied. Also, mixed esters from different acids, *e.g., capsanthin,*[19] have been isolated. Again, crocetin, the coloring matter of saffron, is a digentiobioside [20]—the only carotenoid glycoside so far found—whereas astaxanthin contains the carotenoid bound to protein.

Although little is known of the formation [21] of carotenoids in plants, it appears that synthesis is still able to proceed in the absence of light, as the example of the carrot demonstrates. Moreover, the chlorophyll-free so-called etiolated leaf of maize grown in the absence of light contains carotenoids, although the reverse is true of the etiolated bean leaf.[22] Consideration is given to the pigmentation of autumn leaves later under Xanthophylls.

The color change of ripening fruit from green to yellow or red is frequently due to the disappearance of chlorophyll, which masks the carotenoid present. This is the case with the banana; but often not only the chlorophyll but also the carotenoids disappear, so that an almost colorless phase intervenes which is followed by renewed formation of carotenoids. It is known that carotene-like products are formed by the action of acids on green foliage. The absorption spectra of these products resemble those of carotenoids but they have not the function of vitamin A and their chemistry remains obscure.[22a] Light, atmospheric oxygen, temperature and enzymes are important factors in the natural process, although they act in different directions. The need of oxygen has been repeatedly confirmed, and if, as with the tomato, the temperature is allowed to exceed a certain limit, yellow and not red pigmentation results. The role of the carotenoids in plant metabolism remains obscure, but it is possibly connected with protection of the plant against light.

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[17] Kuhn, Winterstein: *Ber.*, **65,** 646 (1932); **67,** 344 (1934); Kuhn, Brockmann: *Ber.*, **65,** 894 (1932); Kuhn, Grundmann: *Ber.*, **65,** 1880 (1932); Kuhn, Deutsch: *Ber.*, **66,** 883 (1933).

[18] Kuhn, Winterstein, Kaufmann: *Naturwiss.*, **18,** 418 (1930); Zechmeister, v. Cholnoky: *Z. physiol. Chem.*, **189,** 159 (1930); *Ann.*, **481,** 42 (1930).

[19] Zechmeister, v. Cholnoky: *Ann.*, **509,** 269 (1934).

[20] Karrer, Miki: *Helv. Chim. Acta,* **12,** 985 (1929).

[21] *Cf.* the review by Zechmeister in Klein, "Handbuch d. Pflanzenanalyse," **III,** 2, p. 1245, from which the following is largely compiled; see also Zechmeister: "Carotenoide," p. 21.

[22] Willstätter, R., Stoll, A.: "Assimilation der Kohlensäure," p. 134.

α- and β-carotene have the ability [23] to sensitize silver halide emulsions.

The carotenoids [24] found in the animal organism seem, with very few exceptions (*astacin* and similar compounds), to be of vegetable origin. They are encountered in many parts of the body. The connection between *carotene* and *vitamin A* will be considered under Carotene.

The carotenoids occur in plants as constituents of the chromatophors embedded in the plasma.[25] In the green chromatophors their color is masked by the chlorophyll, but those which are free from chlorophyll appear yellow to red in color. The pigment is usually in colloidal solution in the lipoid material, associated with fatty acids or present as colored wax; only rarely is it found in crystalline form (carrot). Their absorption spectra are characteristic, and have frequently afforded material aid in research as well as in determining the concentration of pigments in solution by colorimetric means.[26]

To isolate the pigments—the occurrence of very small amounts of pigments accompanied by large quantities of foreign material is usual in this field—the partition method, first used by Stokes in 1864 and later adopted by Willstätter [27] in his classic researches, is invaluable. The method depends on the principle that the components of a pigment mixture will partition themselves unequally when shaken with a mixture of two immiscible solvents; 70-90 per cent of methyl alcohol, ethyl alcohol or acetone and petroleum or ether are common combinations. Thus hydrocarbons and esters tend to concentrate in the upper phase, while hydroxylated pigments remain preferentially in the lower layer. In such a separation those pigments which concentrate in the lower phase (*e.g.*, alcohol) are termed hypophasic, and those in the upper phase (petroleum) are epiphasic.[28]

[22a] Quackenbush, Steenbock, Peterson, *J. Am. Chem. Soc.*, **60**, 2937 (1938).

[23] Calzavara: *Science Ind. phot. (2)*, **7**, 329 (1936); *Chem. Zentr.*, **1936**, II, 4181.

[24] *See* Zechmeister in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 1245; Zechmeister, "Carotinoide," p. 78.

[25] *See* Zechmeister in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 1255, and Zechmeister. "Carotinoide," p. 78; the cornflower blue color which the pigments give with concentrated sulfuric acid is well known (Marquardt, 1835) but, like the Carr-Price reaction with antimony trichloride, is not specific.

[26] Kuhn, Brockmann: *Z. physiol. Chem.*, **206**, 41, 51 (1932); Ferguson: *Analyst*, **60**, 680 (1935). Theoretical discussion of absorption spectra of carotenoids: Mulliken, *Science*, **89**, 389 (1939); *J. Chem. Physics*, **7**, 364 (1939).

[27] Willstätter, R., and Stoll, A., "Untersuchungen über Chlorophyll," pp. 154, 231; the names of Borodin, v. Krauss and Sorby may be specially mentioned; Kuhn, Brockmann: *Z. physiol. Chem.*, **206**, 41 (1932). A. P. 2031991 (S.M.A. Corp.), *Chem. Zentr.*, **1936**, II, 4238. A. P. 2032006 (S.M.A. Corp.), *Chem. Zentr.*, **1936**, II, 4239.

The further purification of the pigments, and in particular the separation of mixtures of isomerides and closely related compounds, is best effected with the aid of chromatographic adsorption analysis.[29] This consists in allowing a solution to percolate through a column of an adsorbent when the individual compounds, eventually after washing with fresh solvent, occupy separated zones according to their adsorption affinity. The individual zones are separated mechanically and the pigments obtained by elution. A pigment is considered homogeneous only when no further chromatographic separation can be effected.[30] The adsorption affinity among esterified polyene alcohols [31] is dependent not only on the polyene part of the molecule but also on the acyl group. Thus, where a mixture of polyene alcohols has been esterified with one fatty acid, the chromatographic separation has been successful in every case so far attempted. The separation of natural xanthophyll esters is more difficult possibly because the acid constituents merely possess chains of different lengths. Unsaturated acid residues may also account for irregularities, but where such difficulty is encountered the ester may be saponified and the polyene reësterified with a homogeneous acid and then subjected to chromatographic analysis (see capsanthin).

The determination of the constitution of the carotenoids consists first in determining the number of double linkages present, *e.g.*, by hydrogenation,[32] addition of halogens [33] (it was established that, unlike bromine, iodine chloride adds to almost all double bonds [34, 35]) or by addition of oxygen by treatment with perbenzoic acid.[35] Further, hydroxyl groups may be estimated by the method of Zerewitinoff,[36] methoxy groups by Zeisel's method, and carboxyl groups by titration.[37] Methyl side-chains may be detected by oxidation to acetic acid with alkaline potassium permanganate [38] or better by chromic acid.[38a] With the latter, larger fragments[39] may be isolated and a special procedure for this purpose has been devised.[40] With those polyenes which contain ring systems, potassium permanganate affords characteristic degradation products,[41] such as dimethylmalonic, α,α-dimethylsuccinic, geronic and isogeronic acids, providing valuable insight into the original constitution. Finally, degradation with ozone may be effected to yield both large[42] and small[43] oxidation fragments.[41, 42]

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[28] Kuhn, Lederer, Deutsch: *Z. physiol. Chem.*, **220**, 229 (1933). Separation according to Kuhn, Brockmann: *Ibid.*, **206**, 41 (1932).

[29] Tswett: *Ber. deut. bot. Gesell.*, **24**, 316, 384 (1906); **29**, 630 (1911); Kuhn, Lederer: *Naturwiss.*, **19**, 306 (1931); *Ber.*, **64**, 1349 (1931). Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931); Kuhn, Brockmann: *Ibid.*, **200**, 255 (1931) (exhaustive review). Winterstein, Stein: *Ibid.*, **220**, 247, 263 (1933), adsorption series of the carotenoids; review: Zechmeister, v. Cholnoky: *Monatsh.*, **68**, 68 (1936); Isomerization in chromatographic analysis. Gillam, Ridi: *Nature*, **136**, 914 (1935); *Biochemical J.*, **30**, 1735 (1936). Zechmeister, Tuzson: *Ber.*, **72**, 1340 (1939). Zechmeister, v. Cholnoky: "Die chromatographische Adsorptionsanalyse," 2nd Ed., Berlin, 1938. Willstaedt, H.: "L'analyse chromatographique et ses applications," Paris, 1939.

[30] Kuhn, Lederer: *Z. physiol. Chem.*, **200**, 108 (1931).

[31] Zechmeister, v. Cholnoky: *Ann.*, **509**, 269 (1934).

[32] Kuhn, Möller: *Angew. Chem.*, **47**, 145 (1934).

[33] *cf.* Zechmeister, Tuzson: *Ber.*, **62**, 2226 (1929).

[34] Pummerer, Rebmann: *Ber.*, **61**, 1099 (1928).

[35] Pummerer, Rebmann, Reindel: *Ber.*, **62**, 1411 (1929).

[36] Karrer, Wehrli, Helfenstein: *Helv. Chim. Acta*, **13**, 268 (1930).

[37] *cf.* Kuhn, Winterstein, Wiegand: *Ibid.*, **11**, 716 (1928).

[38] Kuhn, Winterstein, Karlowitz: *Helv. Chim. Acta*, **12**, 64 (1929); microdetermination of acetyl, benzoyl and C-methyl groups: Kuhn, Roth: *Ber.*, **66**, 1274 (1933).

[38a] Kuhn, L'Orsa: *Ber.*, **64**, 1732 (1931); *Angew. Chem.*, **44**, 847 (1931); Kuhn, Roth: *Ber.*, **66**, 1274 (1933); Kuhn, Livada: *Z. physiol. Chem.*, **220**, 235 (1933).

Carotenoids may undergo thermal changes which may be referred to one or more of the following processes[44]:

(1) Cyclization without degradation (*e.g.*, tricyclocrocetin).[44]

(2) Formation of monocyclic aromatic hydrocarbons (toluene, *m*-xylene).[45]

(3) Formation of monocyclic aromatic carboxylic acids (*m*-toluic acid from the ends of the chains of carotenoid carboxylic acids, *e.g.*, bixin and azafrin).[46]

(4) Formation of binuclear aromatic hydrocarbons (2,6-dimethylnaphthalene)[47] from correspondingly larger fragments with simultaneous dehydrogenation.

(5) Combination of groups[44] arising according to Scheme 2 (*i.e.*, shortening of the chain).

In all these thermal decompositions the yields are very small. Synthetic work has also played some part in the determination of constitution. For example, perhydronorbixin[48] and perhydrocrocetin[49] and a degradation product of the latter[50] have been synthesized. Determinations of viscosity[51] have also been effected. The absorption of the pigments[52] into the animal organism appears to be selective. Examination of horse serum, lungs, etc. show that the only recognizable carotenoid present is carotene itself, and that ingested plant xanthophylls remain in the intestine.

The known carotenoids are listed in the following table:

[39] Kuhn, Brockmann: *Ber.*, **65**, 894 (1932); **67**, 885 (1934); Kuhn, Grundmann: *Ber.*, 65, 898 (1932).

[40] Kuhn, Brockmann: *Ber.*, **66**, 1319 (1933).

[41] *cf.* Karrer, Helfenstein, Wehrli, Wettstein: *Helv. Chim. Acta*, **13**, 1084 (1930); Karrer, Morf: *Ibid.*, **14**, 1033 (1931).

[42] Karrer, Morf, v. Krauss, Zubrys: *Ibid.*, **15**, 490 (1932).

[43] *cf.* Karrer, Bachmann: *Ibid.*, **12**, 285 (1929).

[44] Kuhn, Winterstein: *Ber.*, **66**, 1733 (1933).

[45] *cf.* van Hasselt: *Chem. Weekblad.*, **6**, 480 (1909); Kuhn, Winterstein: *Ber.*, 65, 1873 (1932); **66**, 429 (1933).

[46] Kuhn, Winterstein: *Ber.*, **65**, 1873 (1932).

[47] Kuhn, Winterstein: *Ber.*, **66**, 429 (1933); see also Kuhn, Deutsch: *Ber.*, 65, 43 (1932).

[48] Karrer, Benz, Morf, Raudnitz, Stoll, Takahashi: *Helv. Chim. Acta*, **15**, 1399 (1932).

[49] Karrer, Benz, Stoll: *Ibid.*, **16**, 297 (1933).

[50] Karrer, Lee: *Ibid.*, **17**, 543 (1934).

[51] Staudinger, Steinhofer: *Ber.*, **68**, 471 (1935).

[52] Zechmeister, Tuzson: *Z. physiol. Chem.*, **1935**, **234**, 235.

Hydrocarbons

Lycopene $C_{40}H_{56}$
Rhodopurpurin $C_{40}H_{56}$ or $C_{40}H_{58}$
α-, β-, γ-Carotene $C_{40}H_{56}$
Leprotene $C_{40}H_{54}$

Xanthophylls

(a) containing one OH-group
  Lycoxanthin $C_{40}H_{56}O$
  Kryptoxanthin $C_{40}H_{56}O$
  Rubixanthin $C_{40}H_{56}O$
  Gazaniaxanthin $C_{40}H_{54-56}O$
  Rhodopin (?) $C_{40}H_{56-8}O$

(b) containing two OH-groups
  Lycophyll $C_{40}H_{56}O_2$
  Rhodoviolascin $C_{42}H_{60}O_2$
  Lutein $C_{40}H_{56}O_2$
  Zeaxanthin $C_{40}H_{56}O_2$
  Eschscholzxanthin (?) $C_{40}H_{54\pm2}O_2$

(c) containing three hydroxyl groups
  Flavoxanthin $C_{40}H_{56}O_3$
  Antheraxanthin (?) $C_{40}H_{58(-2)}O_3$
  Petaloxanthin (?) $C_{40}H_{58(-2)}O_3$
  Eloxanthin (?) $C_{40}H_{56}O_3$

(d) containing four OH-groups
  Violaxanthin $C_{40}H_{56}O_4$
  Taraxanthin $C_{40}H_{56}O_4$

Ketones

Myxoxanthin $C_{40}H_{54}O$
Echinenone $C_{40}H_{58(-2)}O$
Aphanin $C_{40}H_{54}O$
Rhodoxanthin $C_{40}H_{50}O_2$

Hydroxycarbonyl compounds

Capsanthin $C_{40}H_{58}O_3$
β-Citraurin $C_{30}H_{40}O_2$
Capsorubin $C_{40}H_{60}O_4$
Astaxanthin $C_{40}H_{52}O_4$
Fucoxanthin $C_{40}H_{60}O_6$

Carboxylic compounds

Crocetin $C_{20}H_{24}O_4$
Bixin $C_{25}H_{30}O_4$
Azafrin $C_{27}H_{38}O_4$

Compounds in which the function of the oxygen is unknown

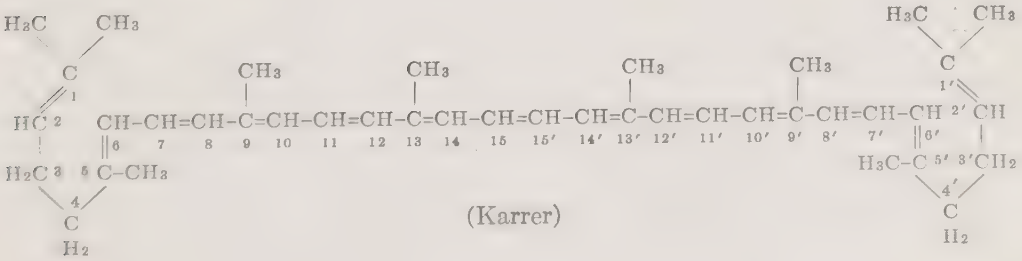
Aphanicin $C_{64}H_{54}O$
Rhodopin $C_{40}H_{58}O$
Rhodoviolascin $C_{40}H_{58}O_2$
Pectenoxanthin $C_{40}H_{52}O_3$
Spirilloxanthin $C_{48}H_{66}O_3$
Pentaxanthin $C_{40}H_{56}O_5$
Asteric acid $C_{40}H_{56}O_6$
Myxoxanthophyll $C_{40}H_{56\pm2}O_7$
Sulcatoxanthin $C_{40}H_{52}O_8$

Pigments characterized by melting points

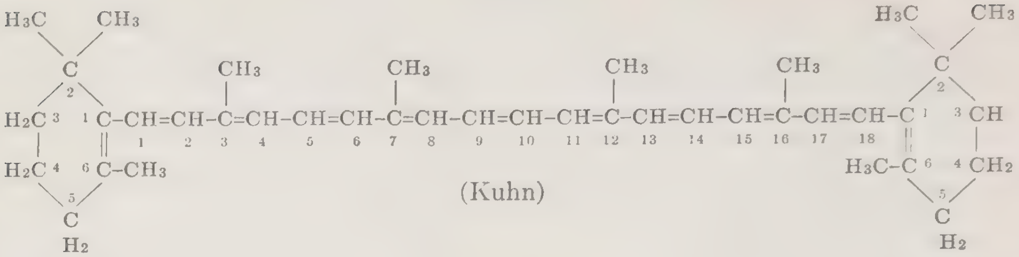
Pigments characterized by absorption spectra

Pigments not further characterized.

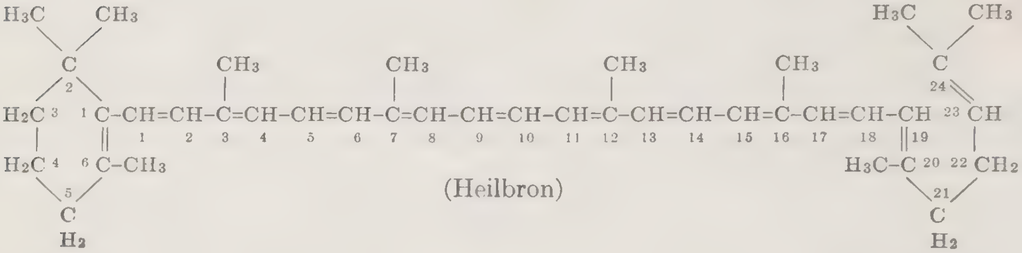
The following examples illustrate systems of numbering carotenoid carbon skeletons:



(Karrer)



(Kuhn)



(Heilbron)

Lycopene is the pigment of the tomato, the fruit of *Lycopersicum esculentum,* and was first isolated in the crystalline form by Millardet [53] who termed it *solanorubin.* Schunck,[54] after other workers [55] had investigated the supposed identity of lycopene with carotene, concluded on the basis of properties and absorption spectra that the pigments are not identical, and proposed the name *lycopene.* A further investigation of Monteverde [56] established the hydrocarbon nature of the pigment, and fundamental results were also obtained by Willstätter and Escher.[57]

Lycopene has also been found in hips (*Rosa canina*),[58] in the ripe fruits of *Tamus communis* (Christmas-rose),[59] in deadly nightshade (*Solanum dulcamara*),[60] in the fruit of the watermelon (*Cucumis citrullus*),[61] in the berries of *Arum maculatum,*[62] in the apricot (*Prunus armeniaca*),[63] in bryony fruit (*Bryonia dioica*),[64] in the golden flowers of the marigold (*Calendula officinalis*),[65] in the fruit of lily-of-the-valley (*Convallaria majalis*),[66] in Kaki fruit (*Diospyros Kaki*),[67] in tropical fruits,[68] in the dark orange blossom of *Dimorphoteca aurantia,*[69] in *Citrus grandis,*[70] in *Passiflora coerulea,*[71] and in bacteria,[72] as in the *thiocystis bactereum.*[73]

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[53] *See* Willstätter, Escher: *Z. physiol. Chem.,* **64,** 47 (1910).

[54] Schunck: *Proc. Roy. Soc. London,* **72,** 165 (1904).

[55] Arnaud: *Compt. rend.,* **102,** 1119 (1886); in connection with the work of Passerini and Kohl see reference 53.

[56] Monteverde: *Staz. sper. agrar. Ital.,* **37,** 909 (1904).

[57] Willstätter, Escher: *Z. physiol. Chem.,* **64,** 47 (1910).

[58] Escher: *Helv. Chim. Acta,* **11,** 752 (1928); Karrer, Widmer: *Ibid.,* **11,** 751 (1928); Matlack: *Am. J. Pharm.,* **100,** 243 (1928).

[59] Zechmeister, v. Cholnoky: *Ber.,* **63,** 422 (1930).

[60] Zechmeister, v. Cholnoky: *Ber.,* **63,** 787 (1930); older literature given.

[61] Zechmeister, Tuzson: *Ber.,* **63,** 2881 (1930).

[62] Karrer, Wehrli: *Helv. Chim. Acta,* **13,** 1104 (1930).

[63] Brockmann: *Z. physiol. Chem.,* **216,** 45 (1933).

[64] Winterstein, Ehrenberg: *Ibid.,* **207,** 25 (1932); give a list of fruits in which lycopene has been detected spectroscopically.

[65] Zechmeister, v. Cholnoky: *Z. physiol. Chem.,* **208,** 26 (1932); Mell: *Textile Colorist,* **57,** 55 (1935).

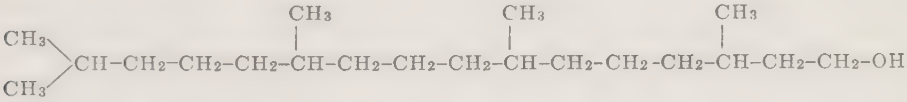
[66] Winterstein, Ehrenberg: *Z. physiol. Chem.,* **207,** 25 (1932).

[67] Karrer, Morf, v. Krauss, Zubrys: *Helv. Chim. Acta,* **15,** 490 (1932).

[68] Zimmermann: *Rec. trav. chim.,* **51,** 1001 (1932); also in the American red and purple tomatoes.

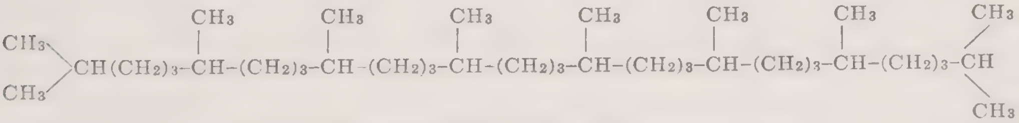
Lycopene ($C_{40}H_{56}$) is distinguished in appearance from the isomeric carotene by its color and crystalline form, forming flattened carmine-red microscopic prisms, m.p. 175°. Solutions in carbon disulfide possess a strong bluish red color; absorption bands in petroleum, (b.p. 70-80°) lie at 506-474-445 mμ. On hydrogenation,[74] the pigment absorbs 13 molecules of hydrogen necessary for the formation of a paraffin hydrocarbon, thus confirming its basically aliphatic structure. Perhydrolycopene ($C_{40}H_{82}$) is an oil (b.p. 238-240° at .03 mm.). The molecular weight experimentally determined agrees with the C_{40} formulation. Lycopene is not attacked by titanium chloride.[75]

The constitution of lycopene may be deduced from the following observations. Dihydrophytol, to which according to Fischer [76] the following constitution must be ascribed,



$$\left.\begin{matrix}CH_3\\ CH_3\end{matrix}\right>CH{-}CH_2{-}CH_2{-}CH_2{-}\overset{\overset{CH_3}{|}}{CH}{-}CH_2{-}CH_2{-}CH_2{-}\overset{\overset{CH_3}{|}}{CH}{-}CH_2{-}CH_2{-}CH_2{-}\overset{\overset{CH_3}{|}}{CH}{-}CH_2{-}CH_2{-}OH$$

was converted into the bromide, and this was treated with potassium, so that two molecules were linked together, affording a hydrocarbon, $C_{40}H_{82}$, of the constitution [77]:



$$\left.\begin{matrix}CH_3\\ CH_3\end{matrix}\right>CH(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}\overset{\overset{CH_3}{|}}{CH}{-}(CH_2)_3{-}CH\left<\begin{matrix}CH_3\\ CH_3\end{matrix}\right.$$

*2-6-10-14-19-23-27-31-Octamethyl-*n*-dotriacontane*

which possessed the same boiling point and physical properties as perhydrolycopene.

Acetone,[78] acetic acid,[79] levulic acid and levulic aldehyde [80] have been isolated from the products of oxidation with ozone; using potassium permanganate, succinic acid [81] has been obtained, and degradation of lyco-

[69] Karrer, Notthaft: *Helv. Chim. Acta,* **15,** 1195 (1932).

[70] Matlack: *J. biol. Chem.,* **110,** 249 (1935).

[71] Karrer, Rübel, Strong: *Helv. Chim. Acta,* **19,** 28 (1936).

[72] Reader: *Biochem. J.,* **19,** 1039 (1925).

[73] Karrer, Solmssen: *Helv. Chim. Acta,* **18,** 1306 (1935).

[74] Karrer, Widmer: *Helv. Chim. Acta,* **11,** 751 (1928); Karrer, Morf: *Ibid.,* **14,** 845 (1931); Karrer, Morf, v. Krauss, Zubrys: *Ibid.,* **15,** 490 (1932); see also p. 493.

[75] Karrer, Helfenstein, Widmer: *Ibid.,* **11,** 1201 (1928).

[76] F. G. Fischer: *Ann.,* **464,** 69 (1928).

[77] Karrer, Helfenstein, Widmer: *Helv. Chim. Acta,* **11,** 1201 (1928); Karrer, Helfenstein, Pieper, Wettstein: *Ibid.,* **14,** 435 (1931).

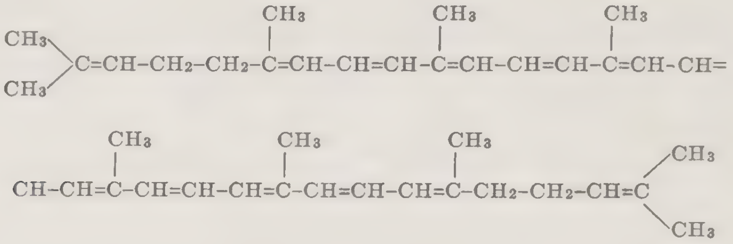
[78] Karrer, Bachmann: *Ibid.,* **12,** 285 (1925); experiments on the addition of alkali metals are also described. Karrer, Helfenstein, Pieper, Wettstein: *Ibid.,* **14,** 435 (1931).

[79] Karrer, Helfenstein, Wehrli: *Ibid.,* **13,** 87 (1930).

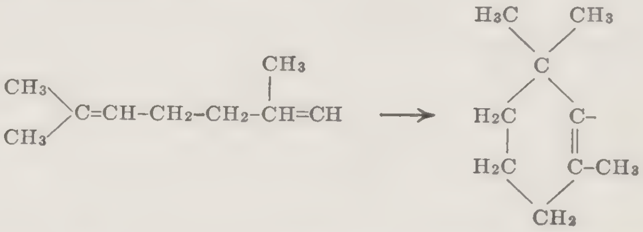
[80] Strain: *J. biol. Chem.,* **102,** 151 (1933).

[81] Winterstein, Ehrenberg: *Z. physiol. Chem.,* **207,** 25 (1932).

pene with chromic acid yields 6 mols. of acetic acid each CH—C(CH_3)= group furnishing 1 mol. of acid. Assuming now the following structure for lycopene:



a relationship between this formula and that of carotene becomes apparent in that the ionone ring of carotene may be elaborated by cyclization of the terminal grouping of lycopene:



This relationship may be compared with the formation of α- and β-ionone from pseudoionone. It may also be mentioned in this connection that the symmetrical formulation discussed under carotene also finds support in the synthesis of perhydrolycopene. The lycopene formula above is, however, confirmed by the products of controlled oxidation with chromic acid.[82] An aldehyde, lycopenal ($C_{32}H_{42}O$, deep-red needles, m.p. 147°), which contains 11 double bonds, is obtained, and the carbon chain split off appears as methylheptenone. Lycopenal, on oxidation with ozone, yields only 0.85 mol. of acetone, corresponding to the fission of one isopropylidene group; the aldehyde group was characterized by the formation of an oxime. Further degradation of lycopenal with chromic acid results in the formation of a second molecule of methylheptenone, together with a mixture of a dialdehyde ($C_{24}H_{28}O_2$, m.p. 220°), and an acid. The aldehyde was converted through a dioxime and dinitrile into a dicarboxylic acid, $C_{24}H_{28}O_4$, which proved to be identical with norbixin (see bixin).

The constitution of norbixin was known from the results of thermal degradation, so that the correctness of the lycopene formula was demonstrated and further confirmation of the constitution of bixin obtained. These reactions are indicated schematically as follows:

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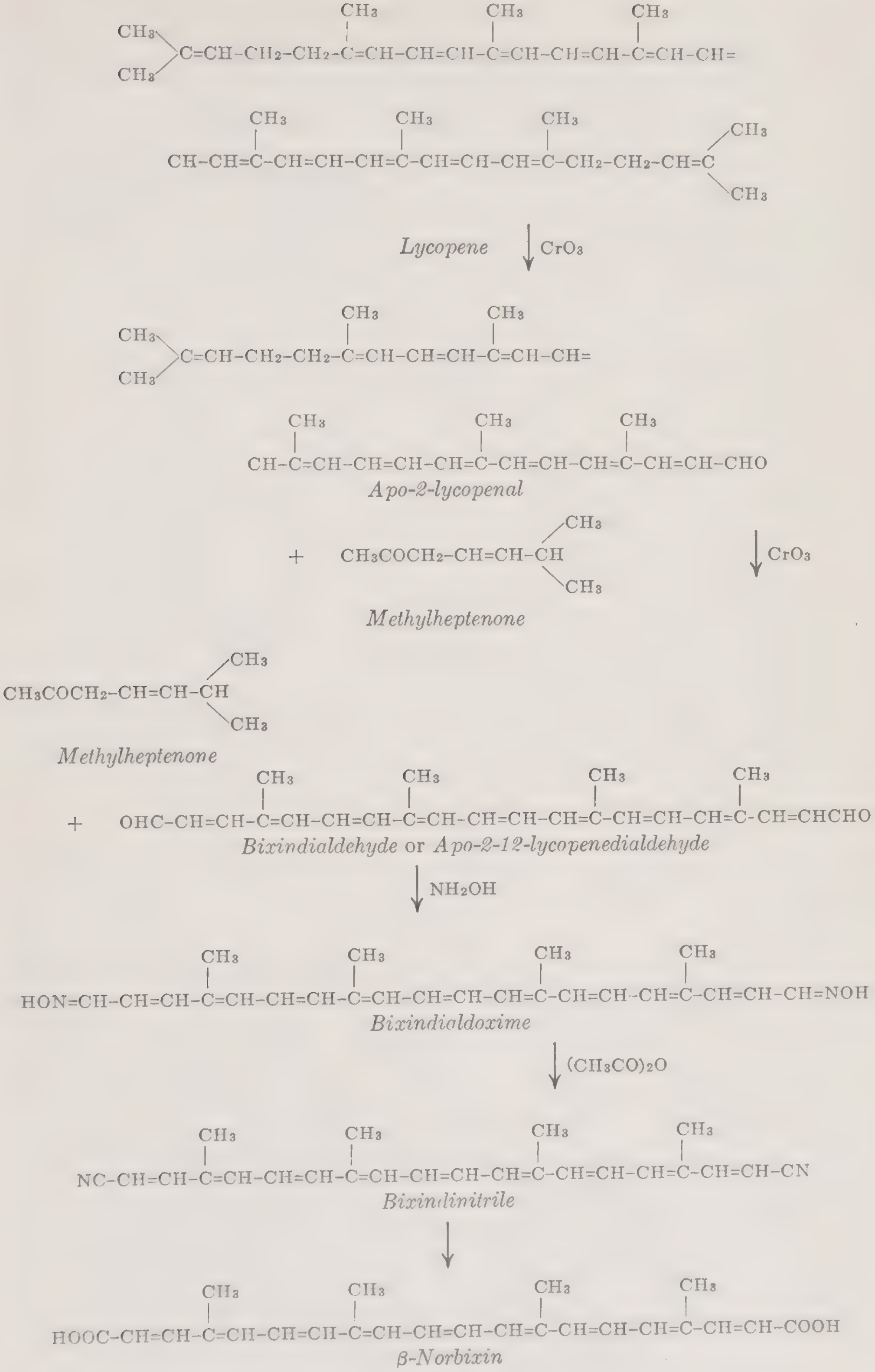
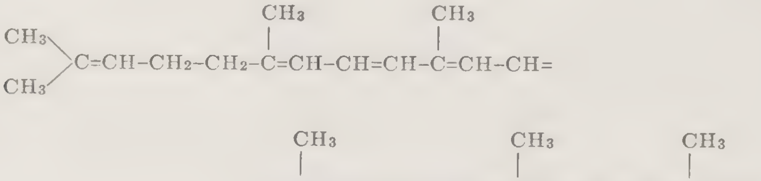
[82] Kuhn, Grundmann: *Ber.*, 65, 898, 1880 (1932); *cf.* Kuhn, Grundmann: *Ber.*, 70, 1905 (1937); Karrer: *Ber.*, 70, 2565 (1937).


Lycopene
CrO₃
Apo-2-lycopenal
+
Methylheptenone
CrO₃
Methylheptenone
+
Bixindialdehyde or Apo-2-12-lycopenedialdehyde
NH₂OH
Bixindialdoxime
(CH₃CO)₂O
Bixindinitrile
β-Norbixin

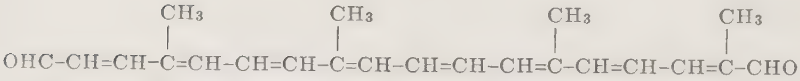
Nomenclature *: Aldehydic fragments obtained by stepwise oxidation of the carotenoids take the name of the parent compound with the suffix "al." The shortening of the chain finds expression in the prefix "apo-" followed by the number of double bonds removed from the parent carotenoid by oxidation.

Two further oxidation products, apo-3-lycopenal ($C_{30}H_{40}O$, brownish crystals, m.p. 140°), and apo-1-bixindialdehyde [apo-3-12-lycopene-dialdehyde, $C_{22}H_{26}O_2$, m.p. 168°, (dioxime, $C_{22}H_{28}O_2N_2$, m.p. $> 210°$] were obtained by mild oxidation with potassium permanganate:[83]



$$\begin{array}{l}\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>C{=}CH{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}\\ \qquad CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CHO\end{array}$$

Apo-3-lycopenal



$$OHC{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CHO$$

Apo-1-bixindialdehyde or *apo-3-12-lycopenedialdehyde*

Thermal decomposition[84] of lycopene yields toluene and *m*-xylene. The formation of lycopene in the plant may be regarded as proceeding from 2 mol. of phytol:

$$2C_{20}H_{40}O = C_{40}H_{56} + H_2O + 11H_2$$

although it is possible that dehydrogenation may take place before the phytol residues become linked together.

Whether lycopene is stereochemically related to β-norbixin (*i.e.*, to the *trans* compound), or whether a *cis-trans* change occurs during the above reactions remains obscure. When treated under conditions which lead to isocarotene, lycopene yields a new pigment which, of all the carotenoid pigments, absorbs farthest toward longer wave-lengths (535-499 mμ in petroleum, b.p. 70-80°). It has also been shown[85] that on standing for 1-2 days while adsorbed on calcium oxide, lycopene undergoes spontaneous isomerization to neolycopene which exhibits absorption bands at 514, 481, 452 mμ (benzene).

Lycopene is most readily obtained from tomato preparations,[86] but while lycopene may be detected in the unripe fruit, the ripe fruit contains also carotene, xanthophylls and xanthophyll esters. Green tomatoes[87] which are ripened at 20-21° assume a color due to lycopene, but at 30° the formation of lycopene is inhibited. Lycopene possesses no growth action.

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* Karrer, Solmssen, Gugelmann: *Ibid.*, 20, 1020 (1937).

[83] Karrer, Jaffé: *Helv. Chim. Acta*, 22, 69 (1939).

[84] Kuhn, Winterstein: *Ber.*, 65, 1873 (1932).

[85] Zechmeister, Tuzson: *Nature*, 141, 249 (1938); similar observation with β-carotene; *cf. Ber.*, 72, 1340 (1939).

[86] Kuhn, Grundmann: *Ber.*, 65, 1885 (1932).

Rhodopurpurin,[88] [$C_{40}H_{56}$ or $C_{40}H_{58}$, needles, m.p. 162°, absorption bands 550-511-479 $m\mu$ (CS_2)], from purple (*rhodovibrio*) bacteria, is possibly identical with lycopene. The positions of the absorption bands indicate 13 double bonds.

Carotene. Berzelius[89] was the first to obtain a yellow coloring matter from autumn leaves, which was accordingly termed leaf-yellow or xanthophyll. In green leaves, however, large quantities of yellow compounds accompany the chlorophyll. In 1885-1887 Arnaud[90] examined a crystalline concomitant of chlorophyll, and found it to be apparently identical with the pigment of the carrot (*Daucus carota*), a conclusion confirmed by Hansen[91] and Monteverde.[91] Carotene was first isolated from the carrot by Wackenroder[91] and more exactly described by Zeise.[92] Analytical results for carotene were first due to Husemann[93] and finally Willstätter and Mieg[91] described a pure preparation assigning the correct formula, $C_{40}H_{56}$. These workers give the melting point as 174°, but later research has shown that a product of this description is a mixture of isomerides; with the aid of methods outlined in the introduction the following isomerides have been obtained in a state of purity:

(1) *α-Carotene,*[94] violet prisms, m.p. 187-188°, $(\alpha)\ \frac{18}{\mathrm{Cd}} = +\,380°$ (benzene), absorption spectrum 478-447.5-423 $m\mu$ (petroleum, b.p. 70-80°).

(2) *β-Carotene,*[94] dark violet prisms, m.p. 184°, $(\alpha)\ \frac{20}{\mathrm{Cd}} = \pm\,0°$ (benzene), absorption spectrum 483.5-452-424 $m\mu$ (petroleum, b.p. 70-80°).

[87] v. Euler, Karrer, v. Krauss, Walker: *Helv. Chim. Acta,* **14,** 154 (1931).

[88] Karrer, Solmssen: *Helv. Chim. Acta,* **18,** 1306 (1935).

[89] Berzelius: *Ann.,* **21,** 257 (1837).

[90] Arnaud: *Compt. rend.,* **100,** 751 (1885); **102,** 1119, 1319 (1886); **104,** 1293 (1887); **109,** 911 (1889); *Bull. Soc. Chim.,* n.s., **48,** 64 (1887).

[91] Willstätter, Mieg: *Ann.,* **355,** 1 (1907); Willstätter, R., and Stoll, A.: "Untersuchungen über Chlorophyll," p. 23, also literature; Rosenberg: *Bull. Soc. Chim. biol.,* **16,** 1761 (1934); Russ. P. 40982 (Rosenberg), *Chem. Zentr.,* **1935,** II, 3833; Russ. P. 48316 (Rosenberg), *Chem. Zentr.,* **1937,** II, 108; U. S. P. 1,967,121 (S.M.A. Corp.), *Chem. Zentr.,* **1935,** I, 273; U. S. P. 1,988,031 (S.M.A. Corp.), *Chem. Zentr.,* **1935,** II, 1405; Matzko: *Chem. Zentr.,* **1938,** I, 4689. Isolation and determination of carotene: Peterson, *Ind. Eng. Chem.* [*Anal.*], **13,** 212 (1941).

[92] Zeise: *Ann.,* **62,** 380 (1847).

[93] Husemann: *Ann.,* **117,** 200 (1861); *Arch. Pharm. (2),* **129,** 30 (1867).

[94] Kuhn, Lederer: *Naturwiss.,* **19,** 306 (1931); *Ber.,* **64,** 1349 (1931); *Z. physiol. Chem.,* **200,** 246 (1931); Kuhn, Brockmann: *Ibid.,* **200,** 255 (1931); Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta,* **14,** 614 (1931); Rosenheim, Starling: Meeting Biochem. Soc. Oxford, May 16, 1931; Karrer, Walker: *Helv. Chim. Acta,* **16,** 641 (1933); Brockmann: *Z. physiol. Chem.,* **216,** 45 (1933); Zechmeister, Tuzson: *Ber.,* **67,** 154 (1934).

(3) γ-*Carotene,*[95] violet prisms, m.p. 178°, $(\alpha)\frac{20}{\mathrm{Cd}} = \pm 0^\circ$ (benzene), absorption spectrum 495-462-431 mμ (petroleum, b.p. 70-80°).

On absorbing β-carotene[96] on alumina or calcium hydroxide, a new pseudo-α-carotene was observed, m.p. 166°, which was optically inactive but spectroscopically identical with α-carotene (507-477 mμ in CS_2); a similar product was obtained by mild oxidation of β-carotene. Similarly the adsorption of α-carotene leads to a new carotenoid with a modified absorption spectrum. This isomerization[97] would now appear to be a general phenomenon which is independent of the chromatographic adsorption, and takes place in solution at room temperature, more rapidly on boiling. A reversible equilibrium is set up, the speed with which this is attained being influenced by catalysts, *e.g.,* iodine. Lycopene, β-carotene, kryptoxanthin, lutein, zeaxanthin and taraxanthin were examined and products, which are to be explained as *cis-trans* isomerides with absorption bands lying toward the shorter wave-lengths, were obtained.

Of the three carotenes, β-carotene may occur almost unaccompanied by the others in nature, whereas α- and γ-carotenes are found as mixtures with the β-isomeride. γ-Carotene is identical with the pigment found by Winterstein and Ehrenberg[98] in *Convallaria majalis* (lily-of-the-valley), and the same compound occurs in the fruits of *Gonocaryum pyriforme* and *obovatum,* together with a δ-carotene[99] which has so far been characterized only spectroscopically (526-490-457 mμ in CS_2). γ-Carotene is responsible for the color of the male gametes of *Allomyces* species.[99a] It occurs also in *Cuscuta salina.*[99b]

The average composition of carotene preparations[100] is 15 per cent of α-, 85 per cent of β-, and 0.1 per cent of γ-carotene, these figures illustrating the extreme delicacy of chromatographic analysis.

The products described in the older literature as *erythrophyll,*[101] *chrysophyll,*[102] *etiolin,*[103] and *xanthocarotin*[104] are probably carotene preparations, but the *chrysophyll* of Hartsen[105] is possibly to be regarded as a xanthrophyll.

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[95] Kuhn, Brockmann: *Naturwiss.,* **21,** 44 (1933); *Ber.,* **66,** 407 (1933); cf. van Stolk, Guilbert, Pénau: *Chimie et Industrie,* **27,** (special number) 550 (1932).

[96] Gillam, El Ridi: *Biochem. J.,* **30,** 1735 (1936); Karrer, Solmssen, Gugelmann: *Helv. Chim. Acta,* **20,** 1020 (1937). Some of the xanthophylls afford several isomerides; three neo-forms of zeaxanthin, for example, have been isolated in crystalline form. See Zechmeister, Cholnoky, Polgar, *Ber.,* **72,** 1678 (1939); also p. 2039, and *Annalen,* **543,** 248 (1940).

[97] Zechmeister, Tuzson: *Ber.,* **72,** 1340 (1939).

[98] Winterstein, Ehrenberg: *Z. physiol. Chem.,* **207,** 25 (1932); Winterstein: *Ibid.,* **215,** 51 (1933); cf. Lubimenko: *Rev. gén. botan.,* **25,** 474 (1914).

[99] Winterstein: *Z. physiol. Chem.,* **219,** 249 (1933).

[99a] Emmerson and Fox, *Proc. Roy. Soc.,* **128B,** 275 (1940).

[99b] Mackinney, *J. Biol. Chem.,* **112,** 421 (1935).

[100] Kuhn. Brockmann: *Ber.,* **66,** 407 (1933); Strain: *J. Biol. Chem.,* **111,** 85 (1935).

[101] Bougarel: *Bull. Soc. Chim.,* n.s., **27,** 442 (1877).

[102] Schunck: *Proc. Roy. Soc. London,* **44,** 448 (1888).

[103] Pringsheim: "Untersuchungen über Chlorophyll," I Abt., Berlin, 1874.

[104] Tschirch: *Ber. Deut. Bot. Gesell.,* **14,** 76 (1896); **22,** 414 (1904).

[105] Hartsen: *Arch. Pharm. (3),* **7,** 136 (1875).

The separation of the carotenes has been effected by fractional crystallization,[106] fractional precipitation with iodine,[107] and by chromatographic separation on silicates,[107] fuller's earth,[108] calcium hydroxide,[109] and activated alumina.[110]

Isocarotene [111] [m.p. 192-193°, violet prisms, absorption spectrum 543-504-427 mμ (CS_2)] is an artificial product formed when the tetraiodide of β-carotene is allowed to stand in benzene, carbon disulfide or acetone solution and then shaken with thiosulfate solution. The conversion is complete within twenty minutes, isocarotene proving to be chromatographically homogeneous. Analysis indicates the formula $C_{40}H_{56}$, but $C_{40}H_{54}$ is not excluded.

Among other physical properties carotene is distinguished by its readiness to crystallize. The color of dilute carotene solutions in most solvents is yellow, resembling aqueous dichromate solutions, whereas more concentrated solutions are deep orange in color. Chemically its character as an unsaturated hydrocarbon is recalled by the gradual bleaching [112] of carotene, a weak odor of ionone being emitted as the crystals absorb oxygen from the air.

The constitution of the isomerides [113] and their interrelationship has been deduced in the following manner. Hydrogenation [114] of carotene—first carried out on the mixture of isomerides—resulted in an absorption of 22 atoms of hydrogen, whereas perbenzoic acid [115] and iodine chloride indicated 8 and 8-11½ double bonds, respectively. However, these last processes yield trustworthy results when used with caution. The experiments were later repeated with pure β-carotene [116] and the influence of the solvent on the hydrogenation was studied. It was established that in place of the paraffin, $C_{40}H_{82}$, a product, $C_{40}H_{78}$, was obtained, and it was deduced that the double linkages are associated with ring systems which would account for the deficiency of four hydrogen atoms.

Further information was gained from oxidations, at first carried out with the natural mixtures and later with the pure isomerides.

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[106] Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta,* **14,** 614 (1931).

[107] Kuhn, Lederer: *Ber.,* **64,** 1349 (1931).

[108] Kuhn, Brockmann: *Z. physiol. Chem.,* **200,** 255 (1931).

[109] Karrer, Morf: *Helv. Chim. Acta,* **16,** 625 (1933).

[110] Kuhn, Brockmann: *Ber.,* **66,** 407 (1933); Strain, *J. Biol. Chem.,* **105,** 523 (1934) *Science:* **79,** 325 (1934); *J. Biol. Chem.,* **127,** 191 (1937).

[111] Kuhn, Lederer: *Naturwiss.,* **19,** 306 (1931); *Ber.,* **65,** 637 (1932); Karrer, Schöpp, Morf: *Helv. Chim. Acta,* **15,** 1158 (1932).

[112] Willstätter, Escher: *Z. physiol. Chem.,* **64,** 47 (1910); H. v. Euler, Karrer, Rydbom: *Ber.,* **62,** 2445 (1929); pure preparations are more stable than impure ones.

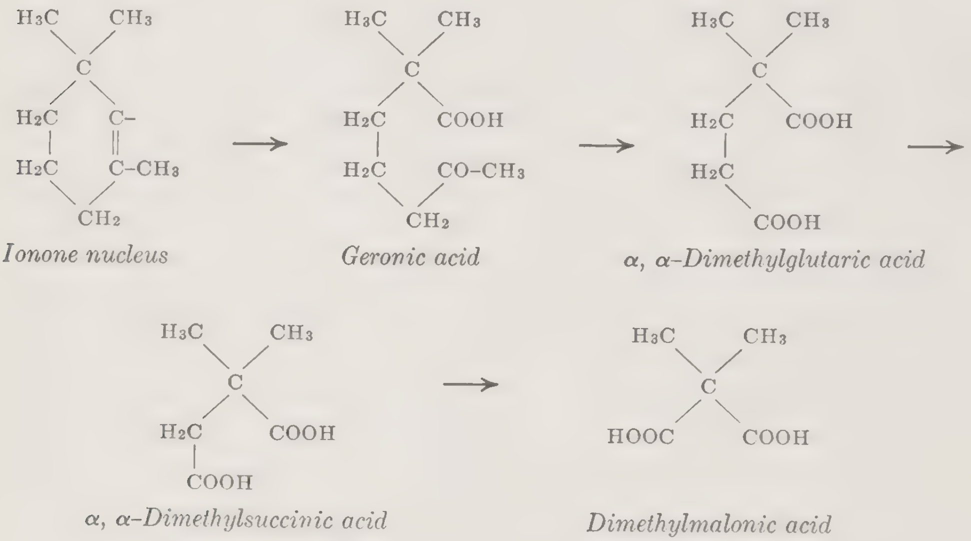
[113] Smith, Milner: *J. Biol. Chem.,* **104,** 437 (1934).

[114] Zechmeister, v. Cholnoky, and Vrabély: *Ber.,* **61,** 566 (1928); Zechmeister, v. Cholnoky: *Ibid.,* **61,** 1534 (1928).

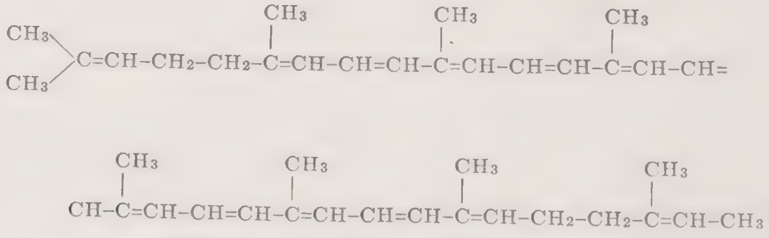
[115] Pummerer, Rebmann: *Ber.,* **61,** 1099 (1928).

[116] Zechmeister, v. Cholnoky, Vrabély: *Ber.,* **66,** 123 (1933); the arguments of Smith: *J. Biol. Chem.,* **96,** 35 (1932) are invalid; see also Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta,* **14,** 614 (1931), particularly p. 623; Karrer, Schöpp, Morf: *Ibid.,* **15,** 1158 (1932), particularly p. 1161; Smith: *J. Biol. Chem.,* **102,** 157 (1933) considers the experimental error.

Ionone [117] and its oxidation products α,α-dimethylsuccinic acid, α,α-dimethylglutaric acid and a little dimethylmalonic acid were early detected, as well as four molecules of acetic acid. Now it was established that, in the oxidation of polyene pigments which are derived from isoprene units, the group $\mathrm{CH{-}\underset{\underset{CH_3}{|}}{C}{=}}$ is completely degraded to acetic acid, and it was concluded that carotene contains a chain of four isoprene units and at last one ionone residue. Geronic acid [118] was then found among the oxidation products, using ozone, so that the following series of degradation products was recognized:



From these results and on the assumption of a close relation between carotene and lycopene, namely that the carotene structure may be derived from that of lycopene by cyclization, the following formulas were suggested:



$$\mathrm{\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>C{=}CH{-}CH_2{-}CH_2{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}}$$

$$\mathrm{CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH_2{-}CH_2{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH_3}$$

Older discarded formula for lycopene

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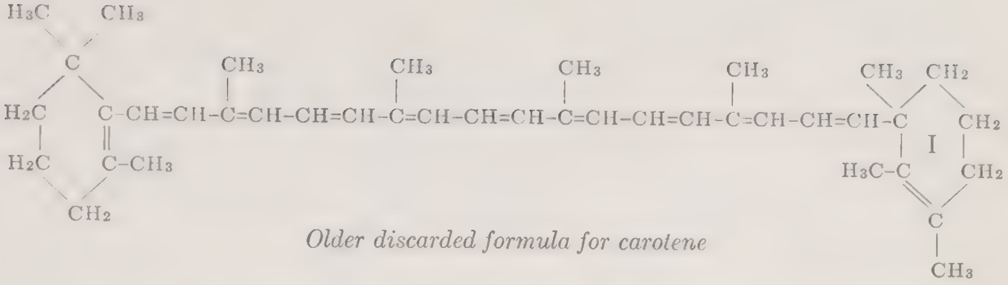
[117] Karrer, Helfenstein: *Helv. Chim. Acta*, 12, 1142 (1929); Karrer, Wehrli, Helfenstein: *Ibid.*, 13, 268 (1930), also p. 270. On oxidation with permanganate see Kuhn, Karlowitz, Winterstein: *Ibid.*, 12, 64 (1929); Karrer: *Ibid.*, 12, 558 (1929).

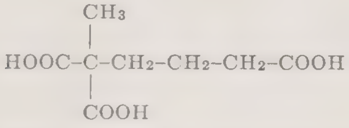
[118] Karrer, Helfenstein, Wehrli, Wettstein: *Ibid.*, 13, 1084 (1930); see also Smith, Spoehr: *J. Biol. Chem.*, 86, 755 (1930); Strain: *Ibid.*, 102, 137 (1933).



Older discarded formula for carotene

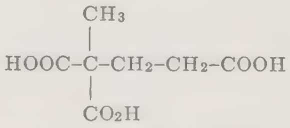
The following facts cannot, however, be reconciled with this formulation:

(1) Oxidation with potassium permanganate of ring I of the carotene formula must result in the formation of pentane-2,2,5- or butane-2,2,4-tricarboxylic acid:



$$HOOC\text{-}\underset{\displaystyle COOH}{\overset{\displaystyle CH_3}{C}}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$$

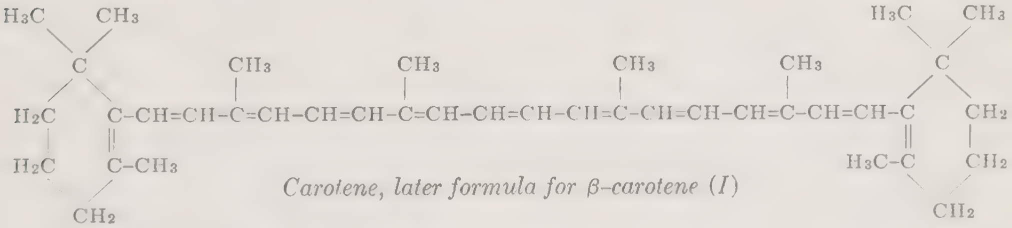
Pentane-2,2,5-tricarboxylic acid



$$HOOC\text{-}\underset{\displaystyle CO_2H}{\overset{\displaystyle CH_3}{C}}\text{-}CH_2\text{-}CH_2\text{-}COOH$$

Butane-2,2,4-tricarboxylic acid

(2) Degradation of the carotene formula[119] requires the production of 5-5.5 mols. of acetic acid, using potassium permanganate, whereas only 4.4 are actually obtained; with chromic acid, 7 mols. would be expected instead of the 6 mols. obtained.[120] So there arises the necessity for formulating the carotene and lycopene formulas so that the terminal groups are symmetrically placed and two (central) methyl substituents stand in the 1,6- instead of the 1,5- relation. This conception has been further confirmed in the case of lycopene.



Carotene, later formula for β-carotene (I)

A further difficulty arose when it was established that carotene consists of a mixture of isomerides, and further that α-carotene is optically active whereas the β-isomeride is inactive. The structure[121] above is assigned to β-carotene as it contains no asymmetric carbon atom, and three further formulas are envisaged which allow optical isomerism:

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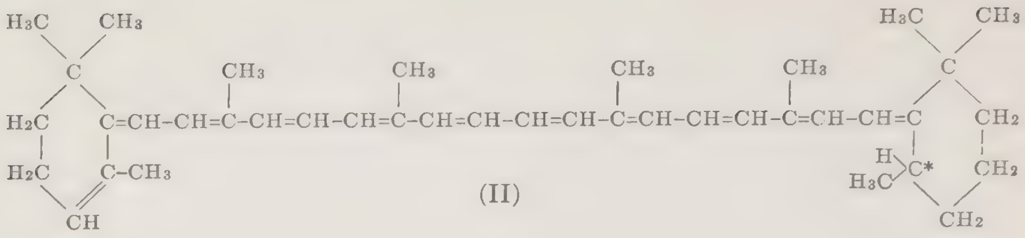
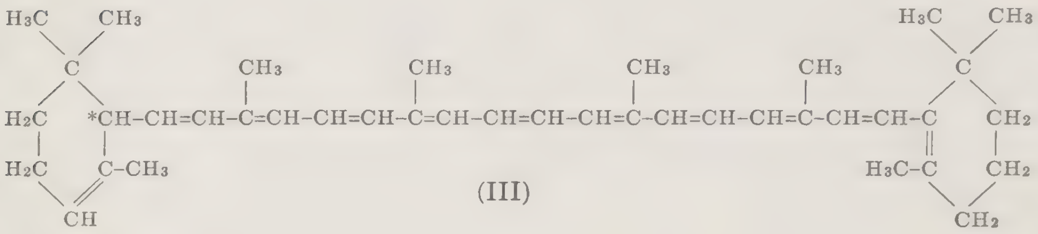
[119] The above formula for lycopene would necessitate 5.5-6 mols. of acetic acid, while only 4.2-4.6 mols. are actually obtained; *see* Karrer, Helfenstein, Wehrli: *Helv. Chim. Acta*, 13, 88 (1930).

[120] The formula for lycopene necessitates 8 mols. acetic acid, while only 6 mols. are actually obtained.

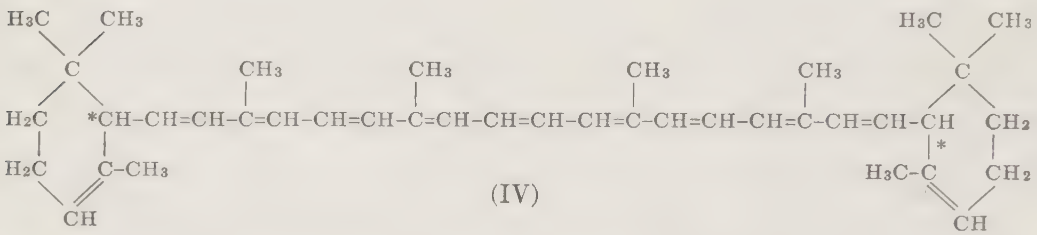
[121] Karrer, Helfenstein, Wehrli, Pieper, Morf [*Helv. Chim. Acta*, 14, 614 (1931)] here refute the views of J. H. C. Smith [*J. Biol. Chem.*, 90, 59 (1931)].



(II)

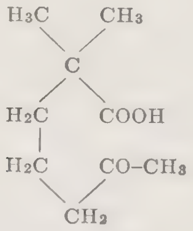


(III)

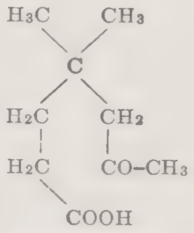


(IV)

On oxidative degradation with potassium permanganate, all four isomerides should yield the same products: α,α-dimethylglutaric acid, α,α-dimethylsuccinic acid, and dimethylmalonic acid, and with ozone I and III should give geronic acid, and II and IV should yield isogeronic acid (see below); III might be expected to yield both.



Geronic acid



Isogeronic acid

Formula I for β-carotene has been substantiated by ozonolysis of a highly purified preparation[122] showing no optical activity when an amount of geronic acid corresponding approximately to two β-ionone rings was isolated.

Finally it has proved possible by controlled use of chromic acid to obtain oxidation products[123] of β-carotene in which the oxidizing agent has attacked only the ends of the conjugated system. The first stage consists in oxidizing the double linkage in one of the ionone rings, with the production of β-oxycarotene[124] ($C_{40}H_{58}O_2$, orange-red needles, m.p. 184°), which is formulated as a glycol:

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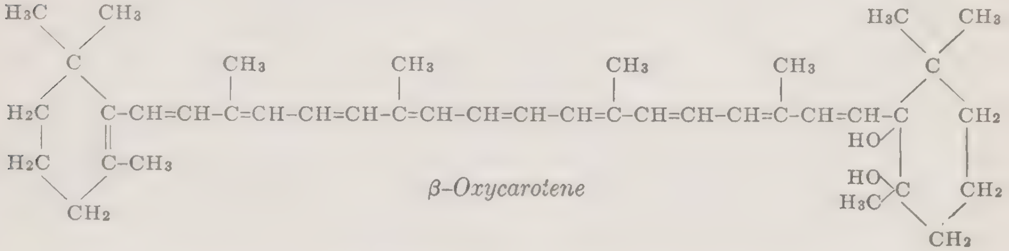
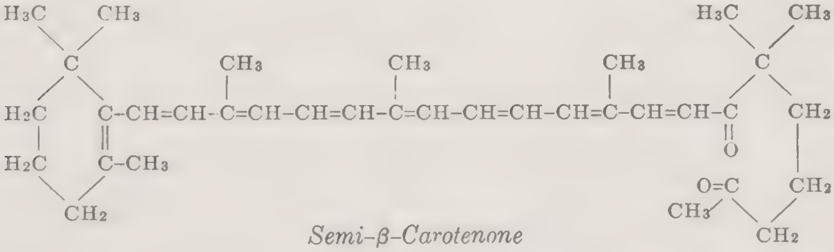
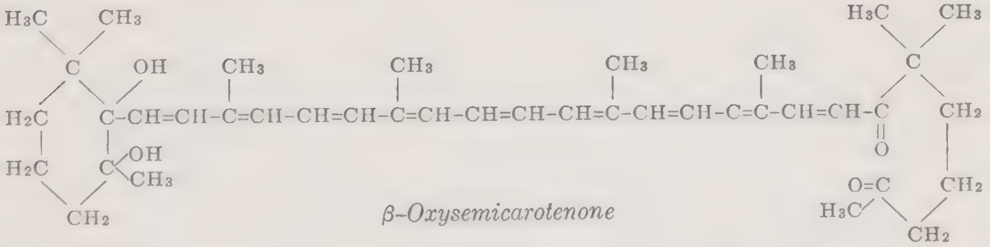
[122] Karrer, Morf: *Helv. Chim. Acta*, 14, 1033 (1931); see also Pummerer, Rebmann, Reindel: *Ber.*, 64, 492 (1931).


β-Oxycarotene

Cautious oxidation of β-oxycarotene with lead tetracetate yielded semi-β-carotenone ($C_{40}H_{56}O_2$, carmine-red, four-sided leaflets, m.p. 118-119°), which may also be obtained directly from β-carotene.[125] Semi-β-carotenone forms a monoxime.[126]


Semi-β-Carotenone

On further gentle oxidation of β-oxycarotene, β-oxysemicarotenone, $C_{40}H_{58}O_4$, is obtained as deep-red prisms (m.p. 172°):


β-Oxysemicarotenone

Both β-oxycarotene and semi-β-carotenone give β-carotenone ($C_{40}H_{56}O_4$, carmine-red leaflets, m.p. 174-175°) on further oxidation, the

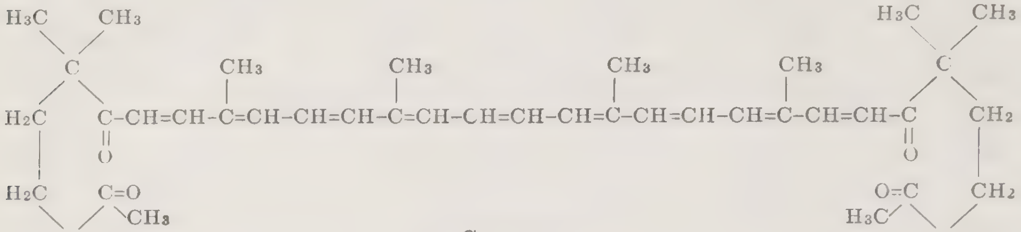
[123] Kuhn, Brockmann: *Ber.*, **65**, 894 (1932); **66**, 833, 1319 (1933); **67**, 1408 (1934); *Ann.*, **516**, 95 (1935); see also Kuhn, Brockmann: *Z. physiol. Chem.*, 213, 1 (1932).

[124] *See* Karrer, H. v. Euler, Solmssen: *Helv. Chim. Acta*, **17**, 1169 (1934); also p. 1171.

[125] *See* Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 25 (1935); Neo-β-oxycarotene, $C_{40}H_{56}O_2$, m.p. 143°, absorption bands 510-479 mμ (CS_2), is also sometimes obtained.

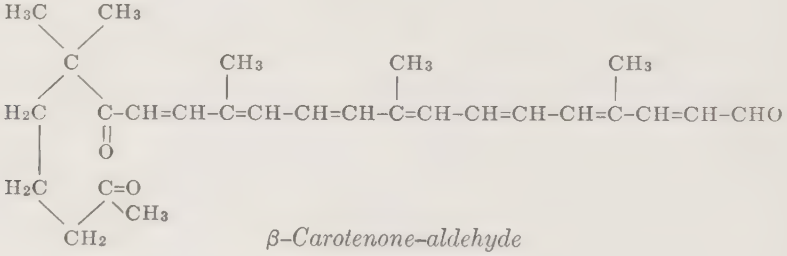
[126] For oxime rules see Kuhn, Brockmann: *Ann.*, **516**, 95 (1935).

same compound being obtained on oxidizing β-carotene directly with the proper amount of chromic acid:



β-Carotenone

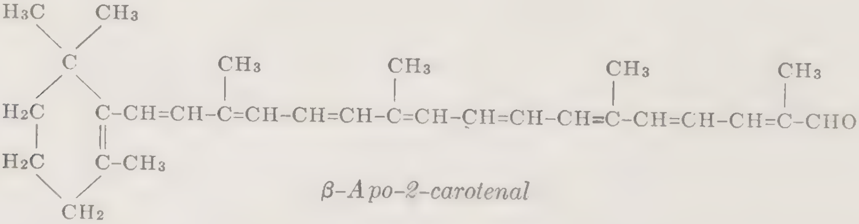
β-carotenone may be further degraded to β-carotenone-aldehyde ($C_{27}H_{36}O_3$, yellow-red needles, m.p. 146-147°), which forms both a monoxime (m.p. 174°) and a dioxime (m.p. 183-184°):



β-Carotenone-aldehyde

Finally still further oxidative degradation of β-carotenone-aldehyde leads to a product [glistening needles, m.p. 110°, absorption bands 436-413 mμ (petroleum)] which is possibly the polyene dialdehyde.

Treatment of β-carotene with potassium permanganate leads to a long-chain aldehyde,[127] β-apo-2-carotenal [$C_{30}H_{40}O$, violet leaflets, m.p. 139°, absorption bands 484-454 mμ (CS_2), (not sharp)]. It forms an oxime, which possesses the biological action of vitamin A, and a semicarbazone, and is given the structure:



β-Apo-2-carotenal

The corresponding alcohol, β-apo-2-carotenol[128] ($C_{30}H_{42}O$, yellow glistening leaflets, m.p. 143°), was obtained by reduction. A further degradation product,[129] also possessing vitamin activity, was isolated only in the form of its oxime, $C_{25}H_{35}ON$ (rhombic leaflets, m.p. 165°) and its semicarbazone, $C_{26}H_{37}ON_3$ (m.p. 217°). The following structure is probable:

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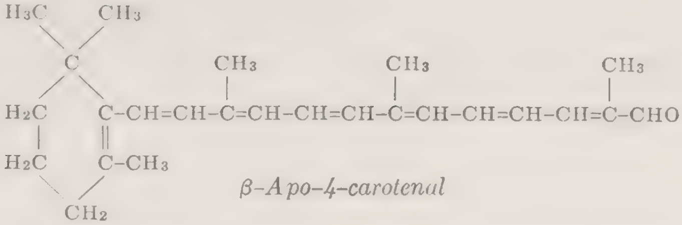
[127] Karrer, Solmssen: *Helv. Chim. Acta*, 20, 682 (1937); the methods of determining the constitution of carotenoids are summarized. v. Euler, Günther, Malmberg, Karrer: *Ibid.*, 21, 1619 (1938).

[128] v. Euler, Karrer, Solmssen: *Ibid.*, 21, 211 (1938).

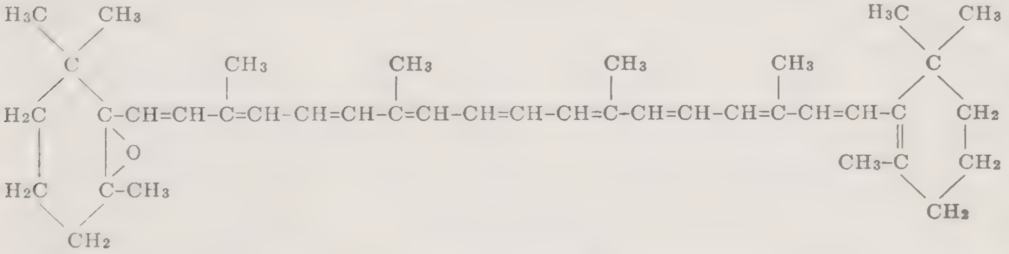
[129] Karrer, Solmssen, Gugelmann: *Ibid.*, 20, 1020 (1937).



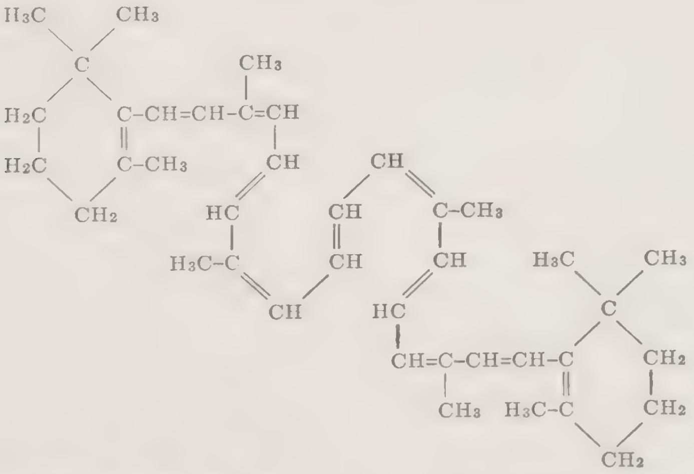
β-Apo-4-carotenal

Oxidation of β-carotene[128] with potassium permanganate also yields β-apo-3-carotenal and a yellow oil which may contain β-apo-5-carotenal.

The close analogy between β-carotenone and rhodoxanthin[130] will be referred to later. On oxidizing β-carotene with perbenzoic acid a carotene oxide[131] (m.p. 161°; the pure compound probably melts higher), was obtained, for which the following formula has been proposed:



Thermal decomposition of β-carotene[132] yields toluene, *m*-xylene and 2,6-dimethylnaphthalene, the formation of which becomes intelligible if the molecule assumes the following configuration:



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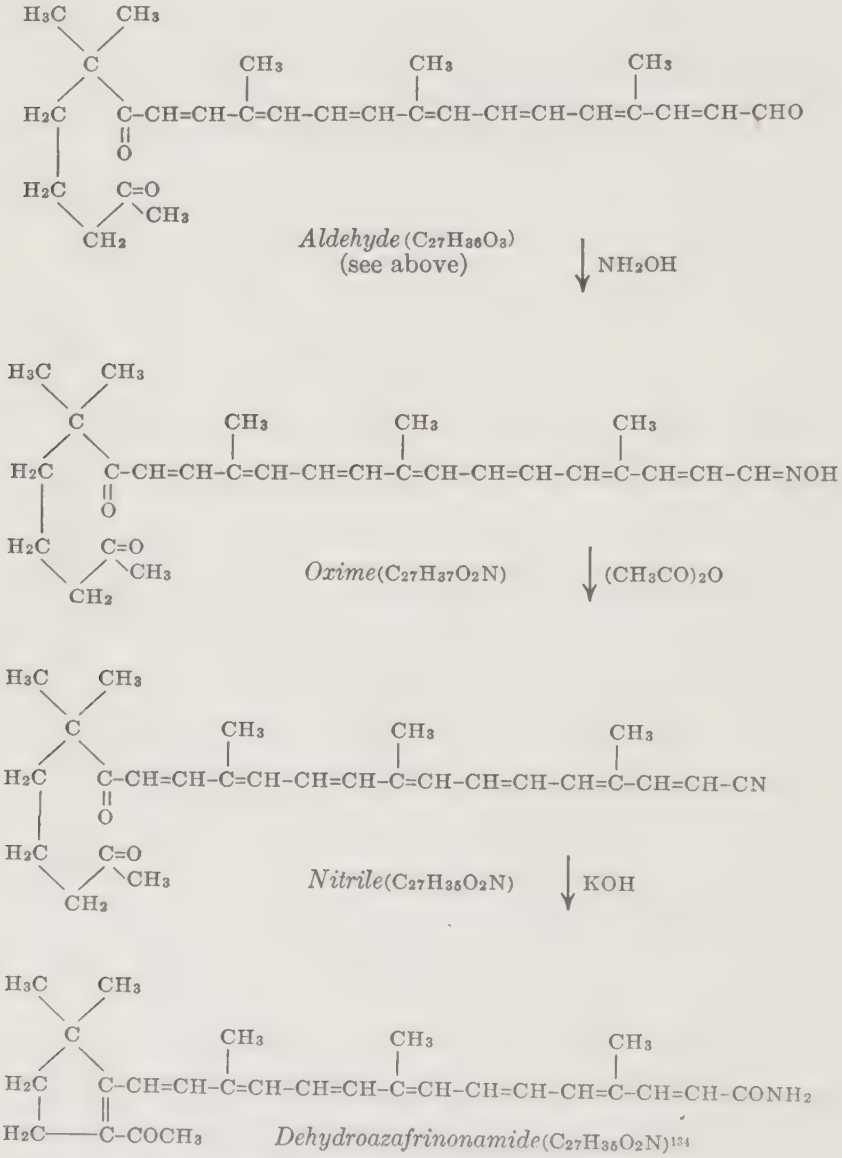
[130] Kuhn, Brockmann: *Ber.*, 66, 828 (1933).

[131] Karrer, H. v. Euler, Hellström, Klussmann: *Arkiv. Kemi, Mineral. Geol., 11B*, No. 3 (1932); v. Euler, Karrer, Walker: *Helv. Chim. Acta*, 15, 1507 (1932).

[132] Kuhn, Winterstein: *Ber.*, 66, 429 (1933); *cf.* the formation of 1,6-dimethylnaphthalene from vitamin A which is biologically and chemically related to β-carotene; Heilbron, Morton, Webster: *Biochem. J.*, 26, 1194 (1932).

This conception provides further support for the correctness of the carotene formula as a considerable part (12 carbon atoms of the whole molecule) remains intact after thermal degradation. Finally the stepwise degradation of β-carotene to dehydroazafrinone amide,[133] which can be obtained from azafrin, has been accomplished, thus mutually confirming the structures assigned to both carotenoids and demonstrating their close connection:



Aldehyde ($C_{27}H_{36}O_3$) (see above)

Oxime ($C_{27}H_{37}O_2N$)

Nitrile ($C_{27}H_{35}O_2N$)

Dehydroazafrinonamide ($C_{27}H_{35}O_2N$)[134]

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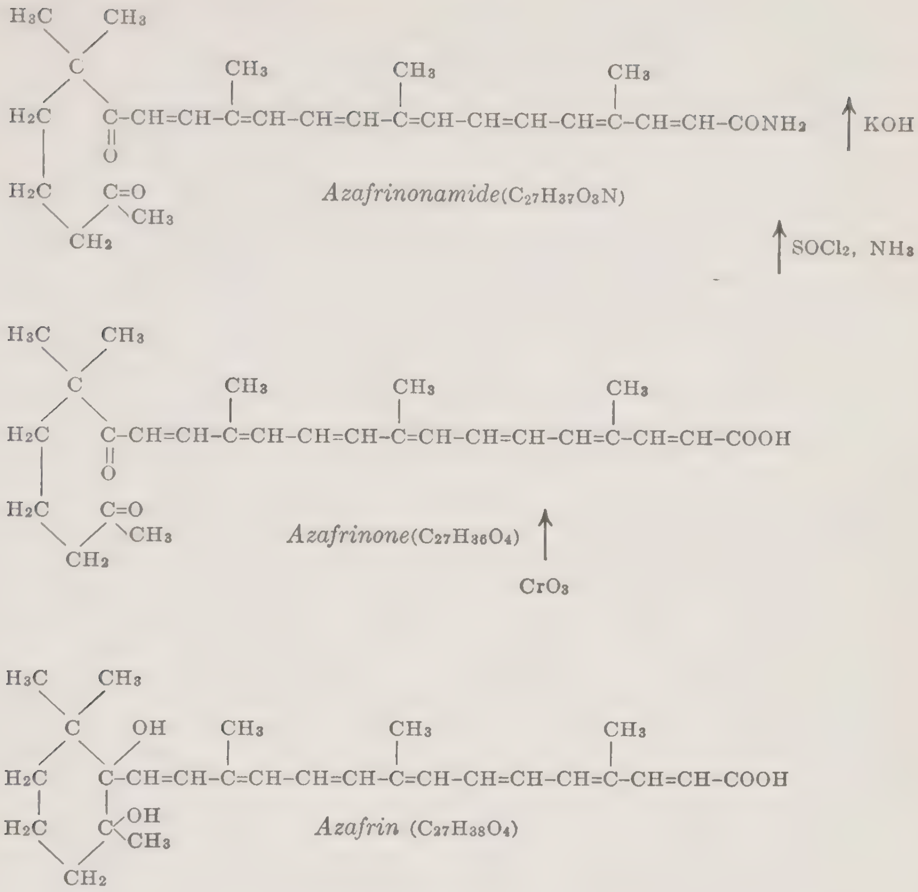
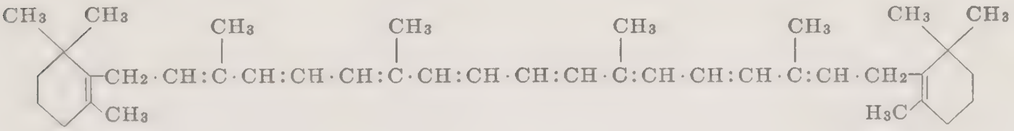
[133] Kuhn, Brockmann: *Ber.*, 67, 885 (1934); *Ann.*, 516, 95 (1935).

[134] This dehydration by alkali is a general property of 1,6-di-ketones such as arise by ring fission of the carotenoid by chromic acid. The resulting dehydrocompounds, which are cyclopentene derivatives, are characterized by their readiness to crystallize and by their absorption bands in the long wave-lengths.


Azafrinonamide ($C_{27}H_{37}O_3N$)
KOH
SOCl₂, NH₃
Azafrinone ($C_{27}H_{36}O_4$)
CrO₃
Azafrin ($C_{27}H_{38}O_4$)

A crystalline dihydro-β-carotene also has been isolated[134a]; it is biologically inactive and its absorption spectrum suggests the structure:

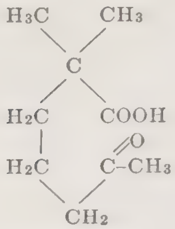


After a fruitless attempt[135] had been made, the constitution of α-carotene was established by the detection of geronic and isogeronic acids among the products of ozonolysis of the pure α-isomeride.[136] The appearance of both acids, corresponding to the terminal groups I and II, indicates the structure III for α-carotene:

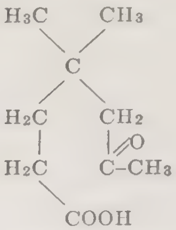
[134a] Karrer and Ruegger. *Helv. Chim. Acta*, **23**, 955 (1940); *cf.* also Euler, Karrer, Hellstrom, Rydbom: *Ibid.*, **14**, 839 (1931).

[135] Karrer, Morf: *Helv. Chim. Acta*, **14**, 833 (1931); Karrer, Morf, v. Krauss, Zubrys: *Ibid.*, **15**, 490 (1932); *cf.* also Kuhn, Lederer: *Ber.*, **64**, 1349 (1931).

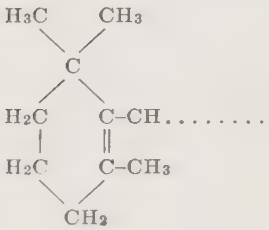
[136] Karrer, Morf, Walker: *Ibid.*, **16**, 975 (1933); *cf.* also *Nature*, **132**, 171 (1933).



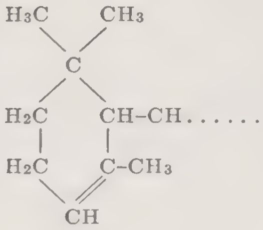
Geronic acid



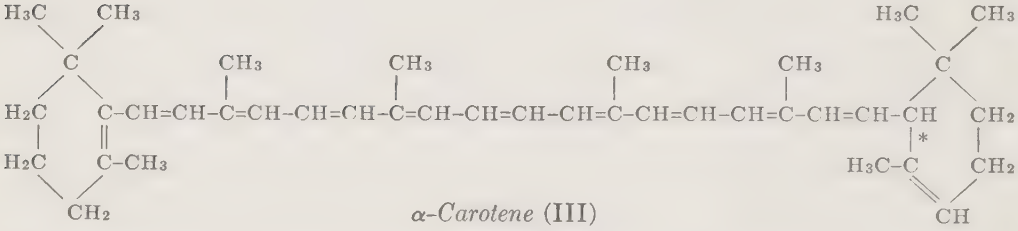
Isogeronic acid



I.

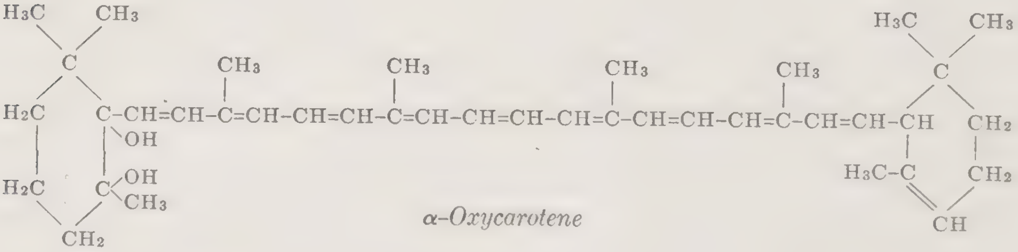


II.



α-Carotene (III)

α-Carotene may be oxidized, by an amount of chromic acid corresponding to 2 atoms of oxygen, to α-oxycarotene [137] (probably $C_{40}H_{50}O_2$, needles, m.p. 183°, absorption bands 502-471-440 mμ), which presumably corresponds to β-oxycarotene. It possesses no vitamin A activity and the following structure [138] is assigned:



α-Oxycarotene

Further degradation leads to α-carotone [$C_{40}H_{56}O_5$, stout prisms with a steely blue reflex, m.p. 148°, $[\alpha]_{644} = 341°$ (±15) (benzene), absorption bands 535 (weak)-502-471 mμ] and a third oxidation product, α-semicarotenone [$C_{40}H_{56}O_2$, needles, m.p. 135°, absorption bands 533-499 mμ (CS_2)], neither of which possesses any vitamin A activity. The latter shows no aldehyde properties, gives a monoxime [139] and is regarded as having the structure:

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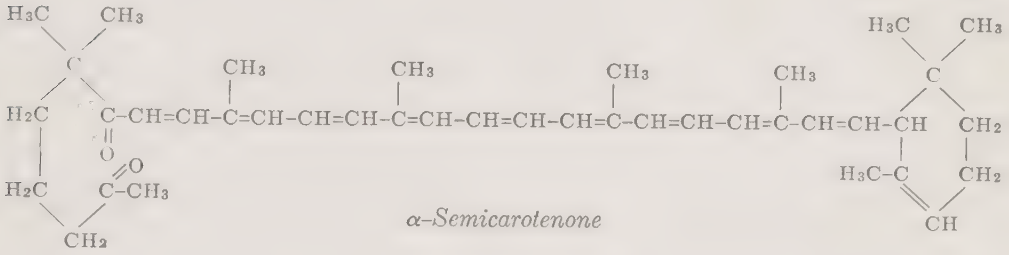
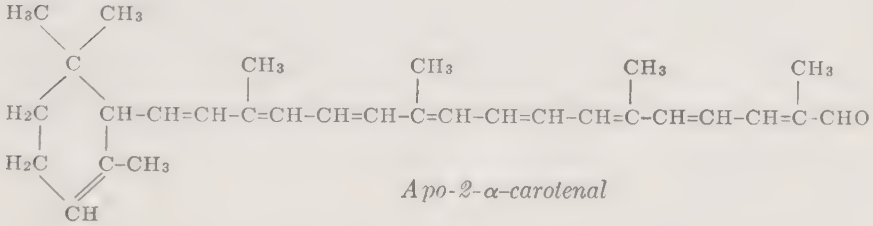
[137] Karrer, Solmssen, Walker: *Helv. Chim. Acta*, **17**, 417 (1934); Karrer was not able to include any consideration of Kuhn's data [Kuhn, Brockmann: *Ber.*, **67**, 1408 (1934)] in this work: Karrer, v. Euler, Solmssen: *Helv. Chim. Acta*, **17**, 1169 (1934).

[138] Kuhn, Brockmann: *Ber.*, **65**, 894 (1932); 67, 1408 (1934); *Z. physiol. Chem.*, **213**, 1 (1932).



α-Semicarotenone

Oxidation of α-carotene [140] with potassium permanganate yields chiefly α-apo-2-carotenal ($C_{30}H_{40}O$, light red crystals, m.p. 158°), without vitamin activity; the oxime ($C_{30}H_{41}ON$, m.p. 178°) forms red clusters of leaflets:



Apo-2-α-carotenal

The aldehyde is reduced to α-apo-2-carotenol ($C_{30}H_{42}O$, light orange crystal masses, m.p. 157°. A higher-melting α-apo-2-carotenal ($C_{30}H_{40}O$, needles, m.p. 174°, oxime, $C_{30}H_{41}ON$, orange prisms, m.p. 185°) was obtained by oxidation of crude carotene; it is possibly related stereochemically to the former isomeride. Theoretically the same products might have arisen as were obtained from β-carotene, but in the unsymmetrical α-isomeride the β-ionone ring is oxidized so that a new series of derivatives emerges.

Unlike α- and β-carotene, γ-carotene [141] absorbs 12 mols. of hydrogen and yields acetone on ozonolysis. In other physical properties also (melting point, position of absorption bands) it takes a position intermediate between lycopene and carotene, and thus is best expressed in the following structure:

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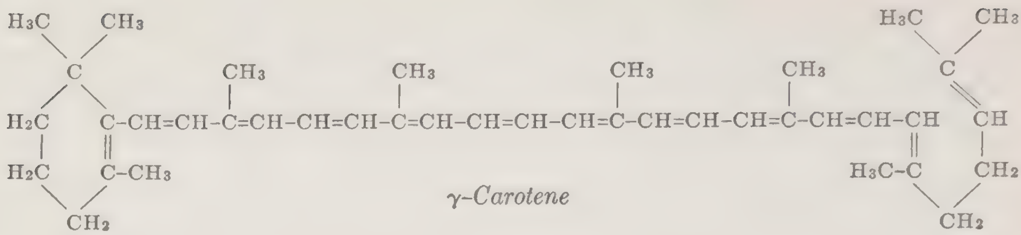
[139] Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 26 (1935).

[140] v. Euler, Karrer, Solmssen: *Ibid.*, **21**, 211 (1938).

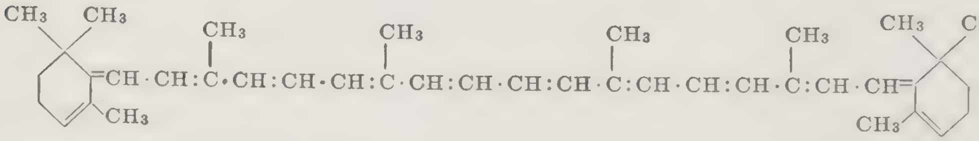
[141] Winterstein, Ehrenberg: *Z. physiol. Chem.*, **207**, 25 (1932); Kuhn, Brockmann: *Ber.*, **66**, 407 (1933).



γ-Carotene

δ-Carotene[142] appears to be a monocyclic pigment derived from α-carotene.

Isocarotene absorbs according to Kuhn[143] 13, according to Karrer[144] 12 mols. of hydrogen on hydrogenation, and yields on ozonolysis no acetone,[143] geronic acid or isogeronic acid[144]; with potassium permanganate,[144] however, α,α-dimethylglutaric, α,α-dimethylsuccinic and possibly succinic acid are formed. Hot permanganate gives four and chromic acid six mols. of acetic acid from which, in conjunction with the formula $C_{40}H_{56}$ and the hydrogenation values, it was concluded that at least one carbocyclic ring, rather than a pure aliphatic structure, is present. More recently it has been confirmed[144a] that isocarotene has the formula $C_{40}H_{54}$ and contains 12 double bonds. The absorption spectrum indicates that these are completely conjugated. Isocarotene has no vitamin activity. No degradation products of the usual type have been obtained, this circumstance rather favoring the formulation of isocarotene as a dehydro-β-carotene:



Curcubitene[145] ($C_{40}H_{56}$), a caretenoid extracted from *Curcubita maxima Duch.* (giant-pumpkin), is β-carotene[146] containing a little α-carotene.

With regard to the occurrence of carotene (mixture) it may be stated that fresh leaves (*e.g.*, of nettles or *Hèracleum*) contain an average of 0.1-0.3 per cent, estimated colorimetrically.[147] Particularly ready sources from which it may be prepared simply and cheaply[148] are the carrot (*Daucus carota*), the skin of the fruit of the paprika (*Capsicum*

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[142] Winterstein: *Z. physiol. Chem.*, **219**, 249 (1933).

[143] Kuhn, Lederer: *Ber.*, **65**, 637 (1932).

[144] Karrer, Schöpp, Morf: *Helv. Chim. Acta*, **15**, 1158 (1932).

[144a] Karrer, Schwab, *Helv. Chim. Acta*, **18**, 477 (1935).

[145] Suginome, Ueno: *Bull. Chem. Soc. Japan*, **6**, 221 (1931).

[146] Zechmeister, Tuzson: *Ber.*, **67**, 824 (1934); see also Winterstein, Ehrenberg: *Z. physiol. Chem.*, **207**, 25 (1932), also p. 27, note 6.

[147] Willstätter, R., and Stoll, A.: "Untersuchungen über Chlorophyll," p. 99, 133, 237.

[148] Zechmeister, in Klein, "Handbuch der Pflanzenanalyse," Vol. **III**, 2, p. 1278; preparation from spinach: U. S. P. 1,953,607 (S.M.A. Corp.), *Chem. Zentr.*, II, **1934**, 2449.

annuum)[149] and the sorb-apple (*Sorbus aucuparia*).[150] The quantitative relation between the isomerides[151] varies considerably in different plants; thus, while β-carotene usually predominates, α-carotene accounts for 30-40 per cent of the total carotene pigment in red palm oil,[152] 25 per cent in the chestnut, 10-20 per cent in the carrot, and 15 per cent in the sorb-apple,[153] whereas only minor proportions occur in the nettle, spinach, grass and paprika.[153] Carotene, not further designated, as the examination of the isomerides present has not yet been carried out, is also found in the ovaries,[154] *corpus luteum* and *rubrum* of the cow,[154] in the human placenta,[155] in gallstones,[156] in animal and human serum,[157] in butter and body-fat, in the suprarenal capsules,[158] in bacteria,[159], etc.

Particular interest is attached to the chemistry of the carotenes by virtue of their relation to vitamin A. It has been shown that α-, β-, and γ-carotene,[160] β-oxycarotene,[161] semi-β-carotenone,[162] β-carotene oxide,[163] and α- and β-carotene diiodides[164] are active, whereas isocarotene,[165] α-oxycarotene, α-carotone, α-semicarotenone [166] and β-carotenone possess no vitamin activity.* Minimum effective doses [167] are 2.5γ of β- and 5γ of α- or γ-carotene, these figures agreeing with the postulated relationship between the carotenes and the vitamin, according to which 1 mol. of α- and γ-carotenes affords 1 mol. of vitamin, whereas 2 mols. may be derived from 1 mol. of β-carotene. The growth action is thus not markedly specific, but is a property of a small group of carotenoid pigments. The activity of the $C_{40}H_{56}$ hydrocarbons and their derivatives is not conditioned merely by the number of double bonds, but is dependent on the presence of at least one ring of the ionone type. The inactivity of isocarotene, however, indicates that further special conditions which at present remain obscure must be fulfilled.[168] *Kryptoxanthin* and *echinenone* among the other natural carotenoids are also active.

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[149] Zechmeister, v. Cholnoky: *Ann.*, **455**, 70 (1927).

[150] Kuhn, Lederer: *Ber.*, **64**, 1349 (1931).

[151] Quantitative estimation: *see* Zechmeister, in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 1285, or Zechmeister, "Carotinoide," p. 117. The content of garden varieties is greater than that of field-cultivated carrots: Bills, Macdonald: *Science*, **76**, 108 (1932); *cf.* Karrer, Schlientz: *Helv. Chim. Acta*, **17**, 7 (1934).

[152] Kuhn, Brockmann: *Z. physiol. Chem.*, **200**, 255 (1931).

[153] Kuhn, Lederer: *Ibid.*, **200**, 246 (1931).

[154] Escher: *Ibid.*, **83**, 198 (1913).

[155] Kuhn, Brockmann: *Ibid.*, **206**, 63 (1932).

[156] H. Fischer, Röse: *Ibid.*, **88**, 331 (1913).

[157] Palmer, Eckles: *J. Biol. Chem.*, **17**, 191, 211 (1914); Palmer, *Ibid.*, **23**, 261 (1915); **27**, 103 (1916); van den Bergh, Muller: *Chem. Zentr.*, **1920**, I, 687; **1920**, III, 720.

[158] Bailly, Netter: *Compt. rend.*, **193**, 961 (1931); Occurrence in *Fusarium* species: Bezssonow: *Compt. rend.*, **159**, 448 (1914); in oils and vegetables: Gill: *J. Ind. Eng. Chem.*, **10**, 612 (1918); Matlack: *Am. J. Pharm.*, **100**, 243 (1928); Schuette, Bott: *J. Am. Chem. Soc.*, **50**, 1998 (1928); Kobayashi, Yamamoto, Abe: *J. Chem. Soc. (Japan)* (Suppl.) **34**, 434B; **35**, 35B (1932); in animal and plant tissues: Zechmeister, Tuzson: *Z. physiol. Chem.*, **226**, 255 (1934); H. v. Euler, Gard, Hellström: *Svensk. Kem. Tid.*, **44**, 191 (1932); in blood serum: B. and H. v. Euler, Hellström: *Ibid.*, **40**, 256 (1928): in banana skins: v. Loesecke: *J. Am. Chem. Soc.*, **51**, 2439 (1929); in oranges: Vermast: *Naturwiss.*, **19**, 442 (1931); Taylor, Witte: *J. Ind. Eng. Chem.*, **30**, 110 (1938); cf. Zechmeister, Tuzson: *Naturwiss.*, **19**, 307 (1931); in mandarine oranges: Zechmeister, Tuzson: *Z. physiol. Chem.*, **221**, 278 (1933); in green tea: Tsujimura: *Sci. Papers Inst. Phys. Chem. Res. (Tokyo)*, **18**, 13 (1932); in fishes: H. v. Euler, Hellström, Klussmann: *Z. physiol. Chem.*, **228**, 77 (1934); in fungae: Willstaedt: *Svensk. Kem. Tid.*, **49**, 318 (1937); Mackinney: *J. Biol. Chem.*, **111**, 75 (1935); Pigmentation of the ripening pumpkin flower: Zechmeister, Béres, Ujhelyi: *Ber.*, **68**, 1321 (1935); pigments of *Genista tridentata:* Schön, Mesquita: *Biochem. J.*, **30**, 1966 (1936); in the pine-apple: Magistad: *Chem. Zentr.*, **1936**, I, 94; in peaty soils: Baudisch, H. v. Euler: *Chem. Zentr.*, **1935**, II, 1390; in pink grapefruits: Matlack: *J. Biol. Chem.*, **110**, 249 (1935); in the sweet potato: Matlack: *Chem. Zentr.*, **1938**, I, 2202; in *Attalea gomphococca:* Blackie, Cowgill: *Food Res.*, **4**, 129 (1939).

[159] Ingraham, Baumann: *J. Bact.*, **28**, 31 (1934).

[160] Kuhn, Brockmann: *Ber.*, **64**, 1859 (1931); A relationship between vitamin A and carotene was first suggested by Steenbock, and the growth action of crystalline carotene was first established by H. v. Euler; H. v. Euler, Karrer, Hellström, Rydbom: *Helv. Chim. Acta*, **14**, 839 (1931); Rosenheim, Starling: *J. Soc. Chem. Ind.*, **50**, 443 (1931); Kuhn, Brockmann: *Ber.*, **66**, 407 (1933); *Z. physiol. Chem.*, **213**, 1 (1932); Winterstein: *Ibid.*, **215**, 51 (1933); Brockmann, Tecklenburg: *Ibid.*, **221**, 117 (1933); v. Euler, Karrer, Zubrys: *Helv. Chim. Acta*, **17**, 24 (1934); Review: Brockmann: *Angew. Chem.*, **47**, 523 (1934); Karrer, Wehrli: "25 years of Vitamin A research," *Nova acta Leopoldina*, **n.F.**, **1**, 175 (1933); Zechmeister: "Lipochrom und Vitamin A," in Schönfeld, Hefter: "Chemie und Technologie der Fette," Vol. **1**, p. 149, Wien, 1936.

[161] Kuhn, Brockmann: *Ber.*, **65**, 894 (1932).

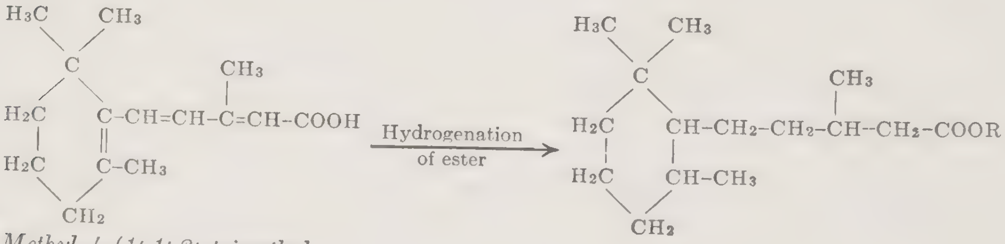
[162] Kuhn, Brockmann: *Ber.*, **66**, 1319 (1933).

[163] H. v. Euler, Karrer, Walker: *Helv. Chim. Acta*, **15**, 1507 (1932).

[164] Karrer, Solmssen, Walker: *Ibid.*, **17**, 417 (1934).

[165] Kuhn, Lederer: *Ber.*, **65**, 637 (1932).

These findings have naturally stimulated interest in the preparation and determination of the constitution of *vitamin A*. Vitamin A, obtained from the liver oil of *Hippoglossus hippoglosus* and from the oil of *Scombresox saurus*,[169] gives geronic acid on degradation. The perhydrovitamin prepared from the natural compound was found to be identical with a synthetic compound [170] prepared according to the following scheme:



2-Methyl-4-(1′,1′,3′-trimethyl 2′, δ²-cyclohexenyl-)butadiene-1-carboxylic acid [171]

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[166] Karrer, H. v. Euler, Solmssen: *Helv. Chim. Acta*, **17**, 1169 (1934).

* Other active carotenoid products are the stereoisomeric forms of α- and β-carotene, α-apo-2- and -4-carotenal and their oximes, the sec. alcohol obtained from α-apo-2-carotenal and ethyl magnesium bromide, myxoxanthin, leprotene, aphanin, and aphanicin. Aphanin has half and aphanicin a quarter of the activity of β-carotene.[166a] More remarkable perhaps is the fact that, although lutein and zeaxanthin are themselves inactive, the products of interaction with phosphorus tribromide are active.[166b]

[166a] Scheunert, Wagner, *Z. physiol. Chem.*, **260**, 272 (1939).

[166b] Euler, Karrer, Zubrys, *Helv. Chim. Acta*, **17**, 24 (1934).

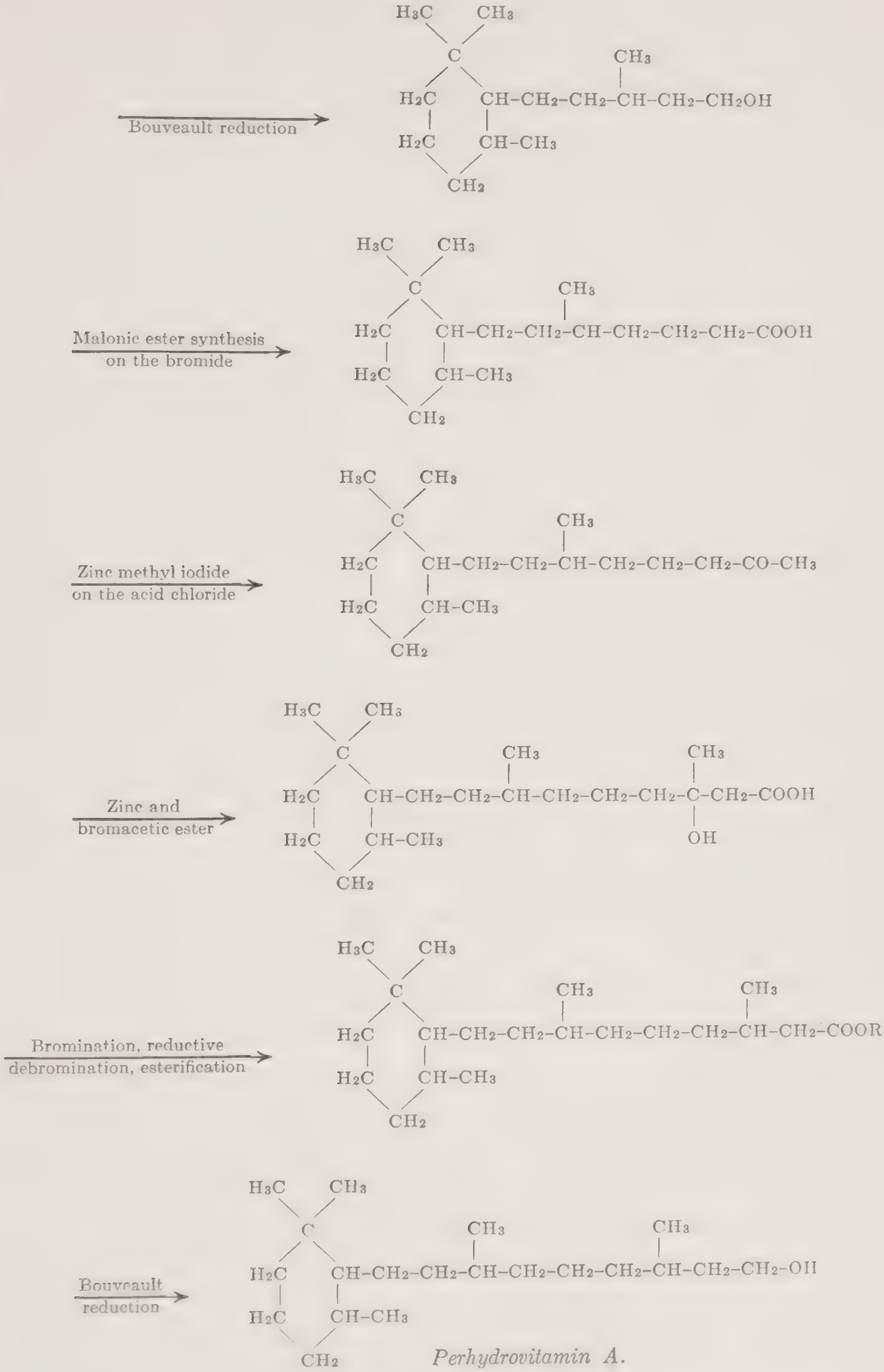
[167] Kuhn, Brockmann: *Klin. Wochenschr.*, **12**, 972 (1933); *Z. physiol. Chem.*, **221**, 129 (1933).

[168] Kuhn, Brockmann: *Ber.*, **66**, 407 (1933).

[169] Karrer, Morf, Schöpp: *Helv. Chim. Acta*, **14**, 1036, 1431 (1931); *cf.* H. v. Euler, Karrer: *Naturwiss.*, **19**, 676 (1931).

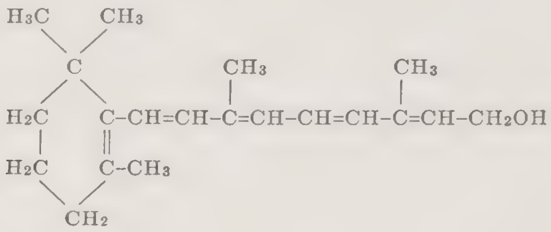
[170] Karrer, Morf, Schöpp: *Helv. Chim. Acta*, **16**, 557 (1933).

[171] Prepared from β-ionone and bromacetic ester: Karrer, Salomon, Morf, Walker: *Ibid.*, **15**, 878 (1932).

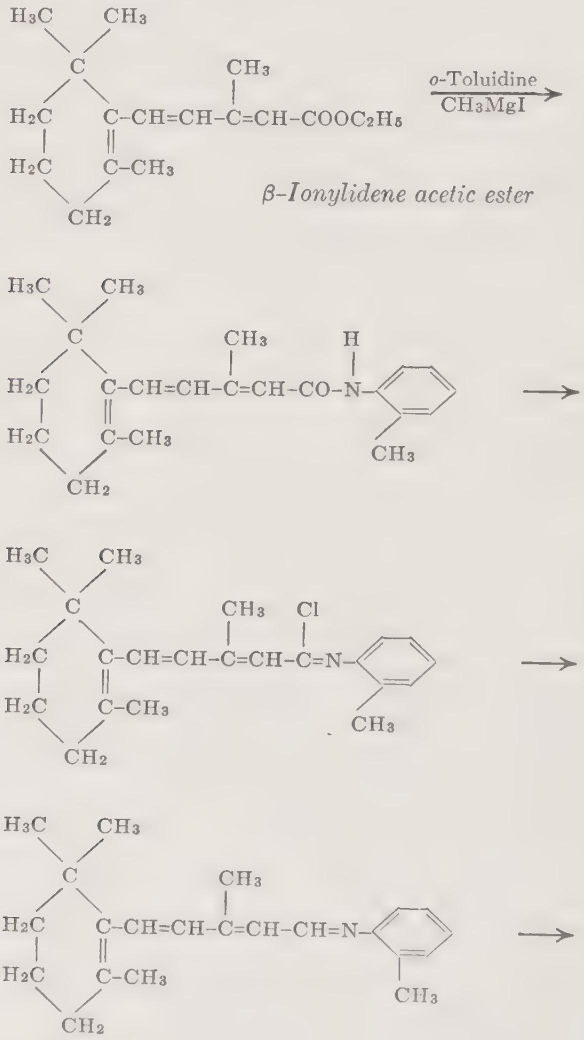

Bouveault reduction
Malonic ester synthesis on the bromide
Zinc methyl iodide on the acid chloride
Zinc and bromacetic ester
Bromination, reductive debromination, esterification
Bouveault reduction

Perhydrovitamin A.

Synthetic perhydrovitamin A, like the product obtained directly from the vitamin, has a boiling point of 148-150° at 0.15 mm. To vitamin A, therefore, was assigned the structure:



which was later synthesized [172] in the following way:


o-Toluidine
CH₃MgI
β-Ionylidene acetic ester

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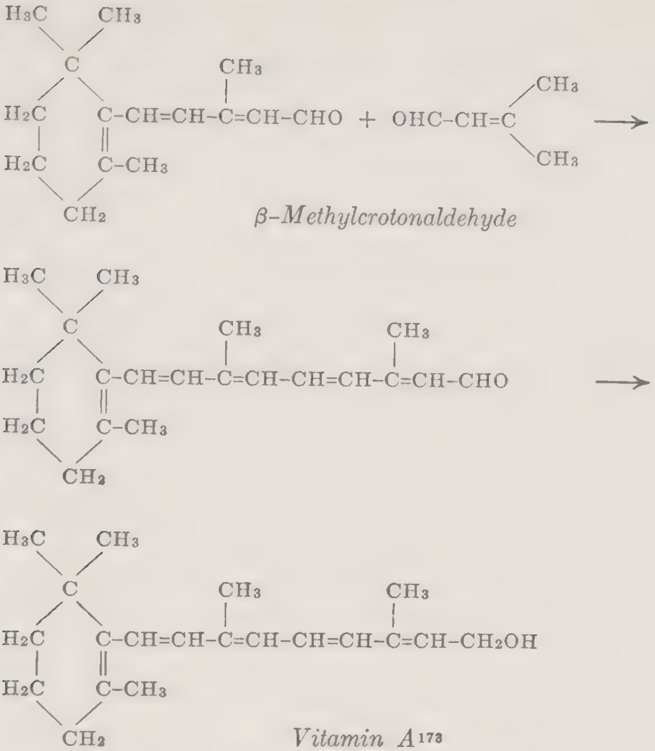
[172] Kuhn, Morris: *Ber.*, 70, 853 (1937).



β-Methylcrotonaldehyde

Vitamin A[173]

Some doubt has been cast on the significance of these reactions as a synthesis [173a]; other workers state that the product of Kuhn and Morris is completely inactive biologically.[173b]

Vitamin A has recently been obtained in crystalline form.[174] The trivial name axerophtol [175] has been suggested for vitamin A. The conversion [176] of carotene into vitamin A *in vitro* has also been effected. Ruzicka [177] was able to isolate 1,6-dimethylnaphthalene from the dehydrogenation of products of ionene, and the same product was obtained by Heilbron [178] from vitamin A.

Finally there is the possibility that an isomeric form [179] of vitamin A is produced from α-carotene:

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[173] Earlier synthetic attempts: Gould, Jr., Thompson, Jr.: *J. Am. Chem. Soc.*, **57**, 340 (1935); Melas, McAlevy: *Ibid.*, **57**, 580 (1935); Davies, Heilbron, Jones, Lowe: *J. Chem. Soc.*, **1935**, 584; F. G. Fischer, Hultzsch: *Ber.*, **68**, 1726 (1935) (elaboration of the carotenoid chain); Heilbron, Jones, Lowe, Wright: *J. Chem. Soc.*, **1936**, 561; Fuson, Christ: *Science*, **84**, 294 (1936), *cf.* Heilbron, Jones: *J. Soc. Chem. Ind.*, **55**, 813 (1936); Batty, Burowoy, Heilbron, Jones, Lowe: *J. Chem. Soc.*, **1937**, 755; D. R. P. 660,621 (Hoffman-La Roche), *Chem. Zentr.*, **1938**, II, 892; Crystalline esters of vitamin A: Hamano: *Ibid.*, **1937**, II, 1209.

[173a] Karrer, Ruegger, *Helv. Chim. Acta*, **23**, 284 (1940).

[173b] Krauze, Slobodin, *J. Gen. Chem. (U.S.S.R.)*, **10**, 907 (1940).

[174] Holmes, Corbet: *J. Am. Chem. Soc.*, **59**, 2042 (1937); U. S. P. 2,111,049 (Parke, Davis & Co.), *Chem. Zentr.*, **1938**, I, 4081.

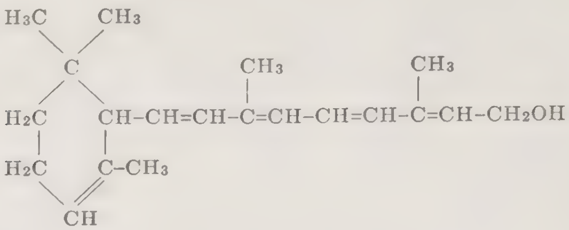
[175] H. v. Euler, Karrer, Solmssen: *Helv. Chim. Acta*, **21**, 211 (1938).

[176] Olcott, McCann: *J. Biol. Chem.*, **94**, 185 (1931).

[177] Ruzicka, Rudolph: *Helv. Chim. Acta*, **10**, 915 (1927).

[178] Heilbron, Morton, Webster: *Biochem. J.*, **26**, 1194 (1932).

[179] Kuhn: *J. Soc. Chem. Ind.*, **52**, 981 (1933); Isomerization: Karrer: H. v. Euler, Solmssen: *Helv. Chim. Acta*, **17**, 1169 (1934); A homolog of vitamin A containing 22 carbon atoms and 5 ethylenic linkages in the side chain (*i.e.*, 6 in all) is discussed by Gillam, Heilbron, Jones, Lederer: *Biochem. J.*, **32**, 405 (1938).



Leprotene.[180] This copper-red pigment ($C_{40}H_{54}$, needles, m.p. 198-200°) was obtained from a strain of acid-resistant bacteria originating in the infectious material from a leper. The absorption bands lie at 517-479-447 mμ (CS_2). From the results of catalytic hydrogenation the hydrocarbon must contain 12 ethylenic bonds. Leprotene may be a provitamin A and thus a dehydro-β-carotene.

Xanthophylls. Carotenoid pigments containing oxygen have been found in many green plant materials, in green and also in yellow leaves, in the skin of the banana,[181] in the dandelion,[182] in the silk cocoon,[183] in the feces of the sheep and cow,[184] and in egg-yolk.[185] Willstätter and Mieg,[186] following many other research workers, showed that such xanthophyll preparation from green leaves have a composition corresponding to the formula $C_{40}H_{56}O_2$. Tswett [187] early suspected the homogeneity of his xanthophyll preparations, and although Willstätter and Stoll were impressed by the similarity between egg-yolk and leaf xanthophylls, they identified themselves with this judgment, and later workers [188] constantly refer to the heterogeneity of xanthophyll preparations.

Kuhn, Winterstein and Lederer,[189] with the aid of chromatographic analysis, were then able to isolate from the known xanthophyll from green leaves, maize, egg-yolk and cow- and sheep-feces two compounds of identical composition but different melting points. It is always possible that others occur in small proportions, as work on such preparations is hindered by the sensitiveness of the xanthophylls toward acids; thus 0.00001*N* oxalic acid was able to depress by 20° the melting point of the lutein later isolated.

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[180] Grundmann, Takeda: *Naturwiss.*, **25**, 27 (1937); Takeda, Ohta: *Z. physiol. Chem.*, **258**, 6 (1939). Leprotene has also been isolated from *Mycobacterium phlei:* Takeda, Ohta, *Z. physiol. Chem.*, **262**, 168 (1939).

[181] v. Loesecke: *J. Am. Chem. Soc.*, **51**, 2439 (1929).

[182] Karrer, Salomon: *Helv. Chim. Acta*, **13**, 1063 (1930).

[183] Oku: *Bull. agri. Chem. Soc. Japan*, **5**, 81 (1930); **6**, 104 (1931).

[184] Karrer, Helfenstein: *Helv. Chim. Acta*, **13**, 86 (1930); *cf.* H. Fischer: *Z. physiol. Chem.*, **96**, 292 (1915).

[185] Willstätter, Escher: *Z. physiol. Chem.*, **76**, 214 (1912).

[186] Willstätter, Mieg: *Ann.*, **355**, 1 (1907); Willstätter, R., and Stoll, A., "Untersuchungen über Chlorophyll," p. 243; Escher: *Helv. Chim. Acta*, **11**, 752 (1928).

[187] Tswett: *Ber. deut. Botan. Ges.*, **24**, 384 (1906), and "Chromophylls in Plant and Animal Kingdom," Warsaw, 1910 (Russ.); see also Palmer, Eckles: *J. Biol. Chem.*, **17**, 191, 211, 223, 237, 245 (1914).

[188] Kylin: *Z. physiol. Chem.*, **163**, 229 (1927); Karrer, Salomon, Wehrli: *Helv. Chim. Acta*, **12**, 790 (1929); Zechmeister, Tuzson: *Ber.*, **62**, 2226 (1929); Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta*, **14**, 614 (1931).

[189] Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).

Kuhn [189] has proposed that the term *xanthophyll* should be reserved as a group name for hydroxylated carotenoids containing 40 carbon atoms, and that the term lutein, which appears in the earlier literature to describe the mixture of egg-yolk pigments, should now be restricted to the compound which is the chief constituent of the xanthophyll mixture of leaves, *i.e.*, to a homogeneous and well characterized preparation. This nomenclature is not however favored by H. v. Euler,[190] who would continue to refer to the egg-yolk mixture as lutein, or by Karrer,[191] who prefers as a collective term for all xanthophylls the expression *phylloxanthin*. Kuhn [192] has, however, retained his nomenclature in agreement with Willstätter, so that the term *xanthophyll* continues to bear the meaning implied by Schunck and Tswett, who had long before emphasized the plurality of the xanthophylls. Kuhn's nomenclature is accordingly employed throughout this volume.

The optically inactive xanthophylls,[193] kryptoxanthin, rubixanthin and flavoxanthin, are derived from inactive hydrocarbons, α-, γ-, and β-carotene by introduction of hydroxyl groups (1, 1, and 2 OH respectively), no optical rotation being observed despite the introduction of new asymmetric carbon atoms by this substitution. Among the dextro-rotatory xanthophylls, lutein is a derivative of α-carotene.[194]

A number of researches [195] have been carried out on the changes taking place in foliage during autumnal fading. The disappearance of chlorophyll is accompanied by the appearance of yellow and red tints. The former are due to carotenoids and their decomposition products, and anthocyanin [196] formation is responsible for the red-violet shades. Tswett [197] substantiated the opinion of previous investigators that, on fading, yellow and colorless substances are produced which are distinguished by giving deep yellow-to-brown solutions with alkali (Nekrobiose). Willstätter and Stoll [198] found that the total carotenoid content of autumn leaves is approximately equal to that of green leaves, but that, while the amount of carotene appears to diminish on fading, the amount of xanthophyll either remains unaltered or increases.[199] The ether-soluble unsaponifiable matter of faded leaves possesses hardly any vitamin A activity,[200] so that no considerable amount of carotene can be present. Tswett also noted that, while the yellow coloring matters were epiphasic like carotene, their adsorption behavior on calcium carbonate recalled that of the xanthophylls; and Tswett therefore termed them autumn xanthophylls (these are the autumn carotenes of Palmer).

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[190] H. v. Euler, Klussmann: *Z. physiol. Chem.*, **208**, 50 (1932).

[191] Karrer, Notthafft: *Helv. Chim. Acta*, **15**, 1195 (1932).

[192] Kuhn, Lederer: *Z. physiol. Chem.*, **213**, 188 (1932).

[193] Kuhn, Grundmann: *Ber.*, **67**, 596 (1934).

[194] Kuhn, Lederer: *Ber.*, **64**, 1349 (1931); Nilsson, Karrer: *Helv. Chim. Acta*, **14**, 843 (1931).

[195] Older literature: L. S. Palmer, "Carotenoids and related Pigments," p. 55.

[196] Berzelius: *Ann. Physik*, **42**, 422 (1837); *Ann.*, **21**, 262 (1837); Wheldale: "The Anthocyanine Pigments of Plants," Cambridge Univ. Press.

[197] Tswett: *Ber. deut. Botan. Ges.*, **26**, 88 (1908).

[198] Willstätter, R., and Stoll, A.: "Untersuchungen über die Assimilation der Kohlensäure," p. 27; *cf.* Goering: *Botan. Zentr. Beihefte*, **35**, 342 (1918).

[199] Immendorf: *Landw. Jahrb.*, **18**, 507 (1889); Kohl: "Untersuchungen über das Carotin und seine physiologische Bedeutung in den Pflanzen." Berlin, 1902; Schunck: *Proc. Roy. Soc. London*, **72**, 165 (1903); Tswett: *Ber. Deut. bot. Gesell.*, **26**, 88 (1908); Roberts: *Chem. Zentr.*, 1938, I, 2203: for a review of the question of autumnal pigmentation, see Bougiovanni, Mones: *Mendel Bull.*, **11**, 26 (1938).

When the xanthophyll esters were discovered it became apparent[201] that they are present in at most only traces in green leaves, but that they appear in considerable quantity at the expense of the free xanthophylls in autumn foliage. The total quantity of xanthophylls and xanthophyll esters undergoes very little change during autumnal fading, so that according to one view, which has not remained undisputed, the natural process consists to a preponderating extent in esterification of the xanthophylls which have been produced in the green-assimilating chloroplasts during spring and summer. Certainly, however, the xanthophylls undergo other modifications, because when they are extracted from the faded leaf only part of the pigment can be isolated in crystalline form. The early changes are probably not deep-seated because, in spite of their lesser readiness to crystallize, the absorption spectrum still agrees closely with that of crystallized lutein; such changes are readily induced artificially by the action of traces of organic acid—the xanthophylls are very sensitive toward acids—and are accompanied by an increase in optical rotation.

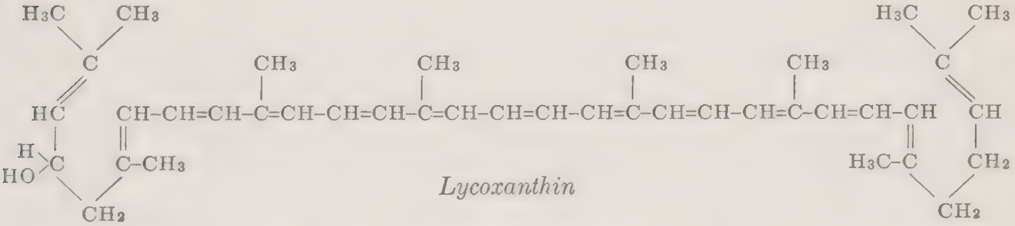
The difficulty experienced in separating pure crystalline carotenoids[202] is due to the occurrence in extracts of faded leaves of extraordinarily large percentages of oily materials of unknown composition, which are not removed by saponification and which possess a remarkable ability to retain carotenoid pigments in solution; the method of separating carotenoids by precipitating with iodine has for this reason proved advantageous. It has been ascertained that on progressive fading of the leaf both carotenes and xanthophylls are destroyed by oxidation, the former disappearing the more rapidly; intermediate decomposition products have not been isolated. Karrer does not subscribe to the view held by Kuhn on the occurrence of larger proportions of esterified xanthophyll in autumn foliage. The autumn xanthophylls of Tswett appear at the commencement of the fading process and increase at the expense of the other carotenoids, so that shortly before the postmortal phase they alone are responsible for the pigmentation of the leaf. They exhibit a spectrum which is not markedly different from that of lutein and present features of the spectrum of violaxanthin. They are epiphasic and may be oxida-

[200] H. v. Euler, Demole, Weinhagen, Karrer: *Helv. Chim. Acta,* **14,** 831 (1931).
[201] Kuhn, Brockmann: *Z. physiol. Chem.,* **206,** 41 (1932).
[202] Karrer, Walker: *Helv. Chim. Acta,* **17,** 43 (1934); Singh, Rao: *Nature,* **140,** 728 (1937).

tion products of the carotenoids, but the question remains open in view of the contradictory results of Kuhn and Karrer.

It would seem that xanthophylls possess growth action [203] only when the formation of vitamin A is possible, as is the case with kryptoxanthin, myxoxanthin and echinenone.

Lycoxanthin [204] ($C_{40}H_{56}O$) forms reddish or dark brown circular clusters, has a melting point of 168° (corr.), and absorption bands of 503-472-443 mμ (petroleum). It occurs together with lycopene in the tomato and in deadly nightshade (*Solanum dulcamara*), and also in the pigment obtained from the fungus *Polystigma rubrium,* from which it may be isolated by chromatographic means. 920 mg. of lycopene and 125 mg. of lycoxanthin were isolated from 17 kg. of nightshade berries. Lycoxanthin acetate forms violet-red needles [m.p. 137° (corr.)] The constitution assigned rests mainly on the similarity between the spectra of lycopene and lycoxanthin. Lycoxanthin may be esterified and behaves as an aliphatic polyene alcohol:



Lycoxanthin

Kryptoxanthin.[205] The pigment occurs in the esterified form to a considerable extent, *i.e.,* one-third of the total pigment, in the calyx and berries of *Physalis* species and is also found in yellow maize [206] and in paprika.[207] It is possible that a pigment, caricaxanthin, probably $C_{40}H_{56}O_2$, which occurs as its palmitic ester [208] in the fruit of *Carica Papaya L.* and also in *Citrus poonensis Hort.*[209] is identical with kryptoxanthin.[210] Its presence in *Physalis* species is easily overlooked, as it is only with difficulty distinguished from β-carotene and is recognized only on analytical [211] examination. Finally, it forms the main pigment of the mandarine orange. Its isolation is effected by chromatographic analysis.

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[203] Karrer, H. v. Euler, Rydbom: *Helv. Chim. Acta,* **13,** 1059 (1930); Klein, Schultze, Hart: *J. Biol. Chem.,* **97,** 83 (1932); H. v. Euler, Karrer, Rydbom: *Helv. Chim. Acta,* **14,** 1428 (1931); Rydbom: *Biochem. Z.,* **258,** 239 (1933); Kuhn, Brockmann: *Z. physiol. Chem.,* **221,** 129 (1933).

[204] Zechmeister, v. Cholnoky: *Naturwiss.,* **23,** 407 (1935); *Ber.,* **69,** 422 (1936), the γ-lycopene of Lubimenko and Brilliant ["Colours of Plants," p. 99, Leningrad, 1924] possibly consists of lycopene admixed with lycoxanthin; Lederer: *Bull. Soc. chim. biol.,* **20,** 611 (1938).

[205] Kuhn, Grundmann: *Ber.,* **66,** 1746 (1933).

[206] Kuhn, Grundmann: *Ber.,* **67,** 593 (1934).

[207] Zechmeister, v. Cholnoky: *Ann.,* **509,** 269 (1934); v. Cholnoky: *Chem. Zentr.,* 1935, II. 230.

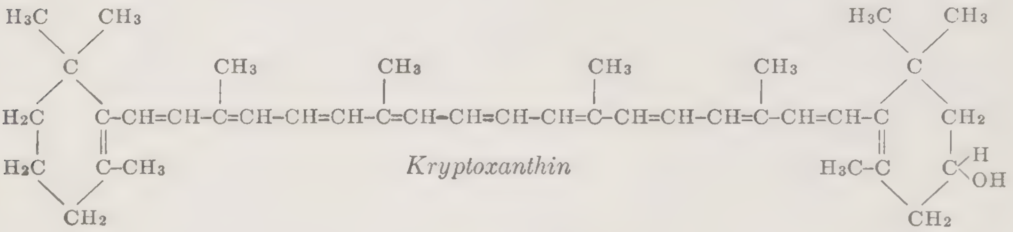
[208] Yamamoto, Tin: *Bull. Inst. Phys. Chem. Research (Tokyo),* **12,** 354 (1933).

[209] Yamamoto, Tin: *Bull. Inst. Phys. Chem. Research (Tokyo),* **12,** 25 (1933); Yamamoto, Kato: *Ibid.,* **13,** 41 (1934).

[210] Karrer, Schlientz: *Helv. Chim. Acta,* **17,** 55 (1934).

[211] Kuhn, Brockmann: *Z. physiol. Chem.,* **206,** 41 (1932).

Its composition, unlike that of caricaxanthin above, agrees with the formula $C_{40}H_{56}O$; the pigment forms prisms with a metallic sheen, m.p. 169°, and is indistinguishable spectroscopically from β-carotene [absorption bands 485,5-452-424 mμ (petroleum, b.p. 70-80°]. Zerewitinoff determinations and the formation of an acetyl compound (garnet-red crystals, m.p. 117-118°) reveal that the oxygen atom is bound as a hydroxyl group. Catalytic hydrogenation results in absorption of 11 mols. of hydrogen, indicating the presence of two rings. These facts agree plausibly with the view that kryptoxanthin is a hydroxy-β-carotene in which one half has the structure of β-carotene and the other that of zeaxanthin:



Kryptoxanthin

The intermediate formulation is reflected in its color and adsorption behavior (β-carotene is not adsorbed, kryptoxanthin weakly and zeaxanthin strongly adsorbed on calcium carbonate). Kryptoxanthin is epiphasic, and passes into the petroleum on shaking with petroleum-90 per cent methanol but, unlike β-carotene, is retained by 95 per cent methanol. The small optical rotation, $[\alpha]\frac{18}{643.5} = \pm 6°$, is however not in agreement with the assumption of an asymmetric carbon atom. While 2 mols. of vitamin A may be derived from β-carotene, it will be noted that the formation of 1 mol. is to be expected from the above formula, and that kryptoxanthin does in fact possess vitamin activity. The striking difference in growth activity between yellow and white maize when fed to rats[213] kept on a diet free from vitamin A depends upon the presence of kryptoxanthin in the yellow variety.

Rubixanthin.[214] The pigment rubixanthin ($C_{40}H_{56}O$, coppery needles, m.p. 160°) is found in hips and haws and is most readily isolated from *Rosa rubiginosa*, less satisfactorily from *Rosa canina* and *Rosa damascena*. It is found also in a Norwegian "Reinrose" and in *Gazania rigens*,[215] and is probably present in the plant in an esterified form. The position of the absorption bands [495.5-463-432 mμ (petroleum b.p. 70-80°)]

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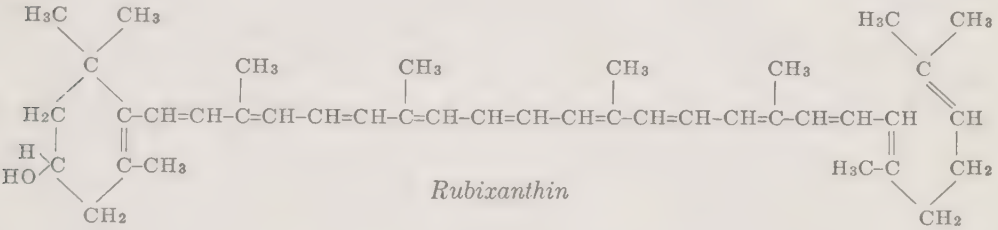
[212] Zechmeister, Tuzson: *Ibid.*, **240**, 191 (1936); Isomerization to neokryptoxanthin: Zechmeister, Tuzson: *Biochem. J.*, **32**, 1305 (1938).

[213] Steenbock, Boutwell: *J. Biol. Chem.*, **41**, 81 (1920).

[214] Kuhn, Grundmann: *Ber.*, **67**, 339, 1133 (1934); Willstaedt: *Svensk. Kem. Tid.*, **47**, 112 (1935).

[215] Schön: *Biochem. J.*, **32**, 1566 (1938).

corresponds to those of γ-carotene, to which the pigment is related. Separation from larger amounts of lycopene and the carotenes is effected by chromatographic analysis, as rubixanthin is the most readily adsorbed on alumina of all epiphasic pigments. In the partition test rubixanthin is epiphasic, but like kryptoxanthin it is retained by 95 per cent methanol. Hydrogenation results, as with γ-carotene, in the absorption of 12 mols. of hydrogen. The oxygen is present in the form of a hydroxyl group (Zerewitinoff determination). Ozonolysis yields acetone, indicating the presence of an isopropylidene group, so that the following formula is assigned:



Rubixanthin

Rubixanthin would seem to be the pigment which Winterstein first observed in *Calendula officinalis.* Rubixanthin containing a hydroxyl group in the ring possesses no vitamin A activity. Remarkable nevertheless is the fact that it shows practically no optical activity, $[\alpha]_{Cd} = \pm 10°$, as does also kryptoxanthin, although it likewise contains an asymmetric carbon atom.

Gazaniaxanthin[216] ($C_{40}H_{54\text{-}56}O$, deep red needles, m.p. 136-137°) was isolated from *Gazania rigens* (absorption bands 530-495.3-463 mμ) and is possibly an isomeride of rubixanthin with the hydroxyl group in the side-chain. It forms an acetate.

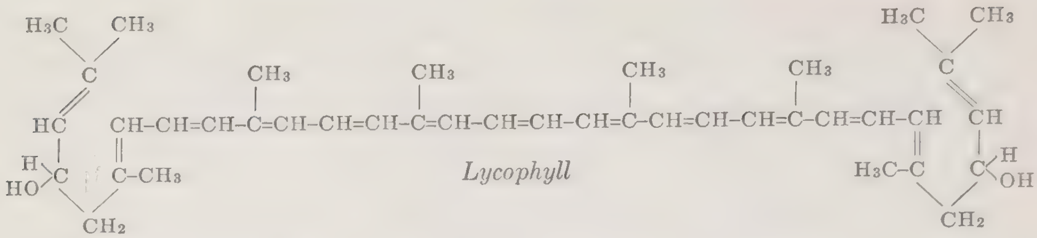
Rhodopin[217] [$C_{40}H_{56\text{-}58}O$, needles, m.p. 171°, absorption bands 547-508-478 mμ (CS_2)] is a carotenoid containing 12 ethylenic linkages and one hydroxyl group which is found in purple (rhodovibrio- and thiocystis-) bacteria.

Lycophyll[218] [$C_{40}H_{56}O_2$, violet-red leaflets or needles, m.p. 179° (corr.), absorption bands 504-473-444 mμ (petroleum)] is present in the tomato and in *Solanum dulcamara*, together with lycopene and lycoxanthin from which it is separated chromatographically. It forms a dipalmitate, m.p. 76°, and the following structure expresses its behavior as a purely aliphatic polyene alcohol:

[216] Schön: *Biochem. J.*, **32**, 1566 (1938).

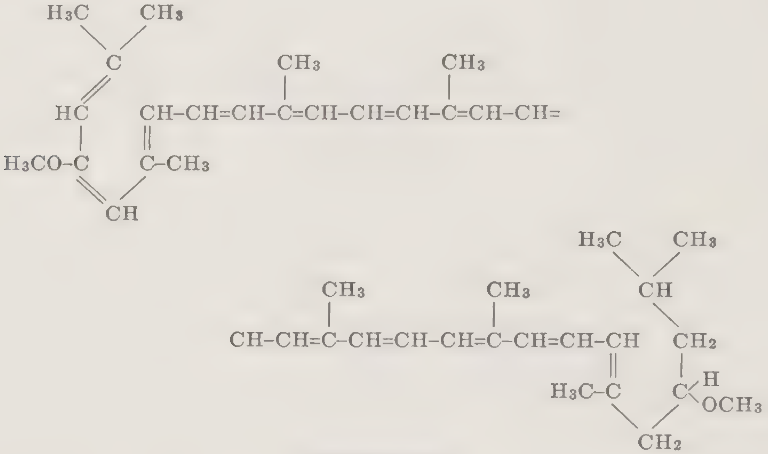
[217] Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 1306 (1935); **19**, 1019 (1936); Karrer, Solmssen, Koenig: *Ibid.*, 21, 454 (1938).

[218] Zechmeister, v. Cholnoky: *Naturwiss.*, **23**, 407 (1935); *Ber.*, **69**, 422 (1936).



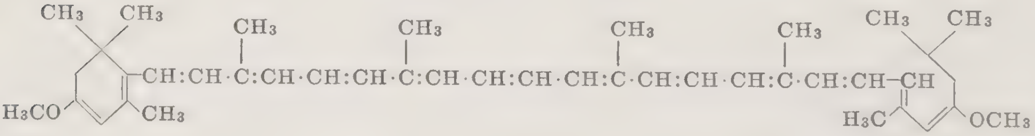
Lycophyll

Rhodoviolascin[219] ($C_{42}H_{60}O_2$, spindle-shaped crystals, m.p. 218°, absorption bands 573-534-496 mμ) has been obtained from rhodovibrio- and thiocystis-bacteria. It contains 13 double linkages and 2 methoxyl groups and possibly possesses the following formula as an open-chain carotenoid:



Rhodoviolascin

More recently a monocyclic formulation has been suggested[219a]:



Lutein[220] [$C_{40}H_{56}O_2$, yellow-red shining prisms, m.p. 195°, $[\alpha]\frac{18}{\mathrm{Cd}} = +165°$ (benzene)[221] absorption bands 477.5-447.5 mμ (petroleum b.p. 70-80°)] occurs in egg-yolk together with zeaxanthin, the name having been originally applied to the mixed egg-yolk pigments.[222] It is widely distributed in the plant kingdom, *e.g.*, in grass,[223] in the leaves of the horse-chestnut, nettle, meadow clover, spinach, in yellow maize,[224] and in many yellow flowers such as *Tagetes, Helenium, Helianthus,*[225] the sunflower,[226] dandelion,[227] and many others.[228] It has not yet been established to what extent lutein occurs as an ester in these plants. Tswett's [229] xanthophyll and Schunck's [230] xanthophyll are identical with lutein. Hydrogenation of a mixture of isomerides [231] (nettle preparation, m.p. 172°) resulted in an uptake of 11 mols. of hydrogen; and a Zerewitinoff determination on the same specimen [232] revealed that both the oxygen atoms are present as hydroxyl groups. Thus it may already be conconcluded from these two observations that two rings are present, as in carotene. Any remaining doubt that the oxygen atoms were linked as hydroxyl groups was removed when xanthophyll esters were found in nature [233] and when it was found possible to esterify one [234] or both [235] of the hydroxyls in the laboratory. Degradation of a xanthophyll preparation with potassium permanganate [236] yielded α,α-dimethylsuccinic acid but no α,α-dimethylglutaric acid. This would be explained if the methylene group at C_3, which appears in the case of carotene as the α′ carbon atom in α,α-dimethylglutaric acid, were replaced in xanthophyll by the group CH—OH, in which case oxidation would proceed directly through dimethylhydroxyglutaric acid to dimethylsuccinic acid:

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[219] Karrer, Solmssen: *Helv. Chim. Acta,* **18,** 1306 (1935); older literature, particularly of α- and β-bacteriopurpurin: *Helv. Chim. Acta,* **19,** 3, 1019 (1936).

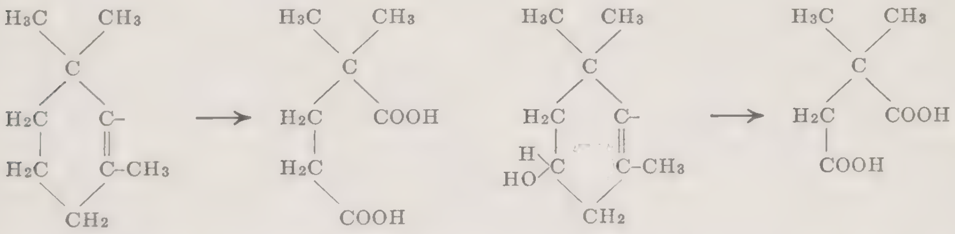
[219a] Karrer, Solmssen, Koenig, *Helv. Chim. Acta,* **21,** 454 (1938); Karrer, Koenig: *Ibid.,* **23,** 460 (1940).

[220] Nomenclature: Kuhn, Lederer: *Z. physiol. Chem.,* **213,** 188 (1932); also p. 191.

[221] Kuhn, Grundmann: *Ber.,* **67,** 596 (1934).

[222] Kuhn, Lederer: *Z. physiol. Chem.,* **213,** 188 (1932).

[223] Kuhn, Winterstein: *Naturwiss.,* **18,** 754 (1930).



Although Karrer considered the possibility of there being only one carotene ring, the presence of two is now accepted, but they do not possess the same fine structure. Again the presence of hydroxyl groups in position 4 would be reflected in enolic properties for which there is no experimental justification. Oxidation of a perhydrolutein yielded a diketone,[237] presumably of the formula:

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[224] Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).
[225] Zechmeister, Tuzson: *Ber.*, 63, 3203 (1930).
[226] Zechmeister, Tuzson: *Ber.*, **67**, 170 (1934).
[227] Karrer, Salomon: *Helv. Chim. Acta*, 13, 1063 (1930).
[228] Karrer, Notthafft: *Ibid.*, **15**, 1195 (1932); Kuhn, Winterstein: *Ber.*, **64**, 326 (1931).
[229] Tswett: *see* Kuhn, Brockmann: *Z. physiol. Chem.*, **206**, 60 (1932). The chrysophyll of Hartsen [*Arch. Pharm. (3)*, 7, 136 (1875)] is probably a xanthophyll.
[230] Schunck: *Proc. Roy. Soc. London*, 72, 165 (1904).
[231] Zechmeister, Tuzson: *Ber.*, 61, 2003 (1928).
[232] Karrer, Helfenstein, Wehrli: *Helv. Chim. Acta*, 13, 87 (1930).
[233] Kuhn, Winterstein, Kaufmann: *Naturwiss.*, 18, 418 (1930).
[234] Karrer, Jirgensons: *Helv. Chim. Acta*, 13, 1102 (1930).
[235] Karrer, Ishikawa: *Ibid.*, 13, 709, 1099 (1930).
[236] Karrer, Wehrli, Helfenstein: *Ibid.*, 13, 268 (1930).
[237] Karrer, Zubrys, Morf: *Helv. Chim. Acta*, 16, 977 (1933).

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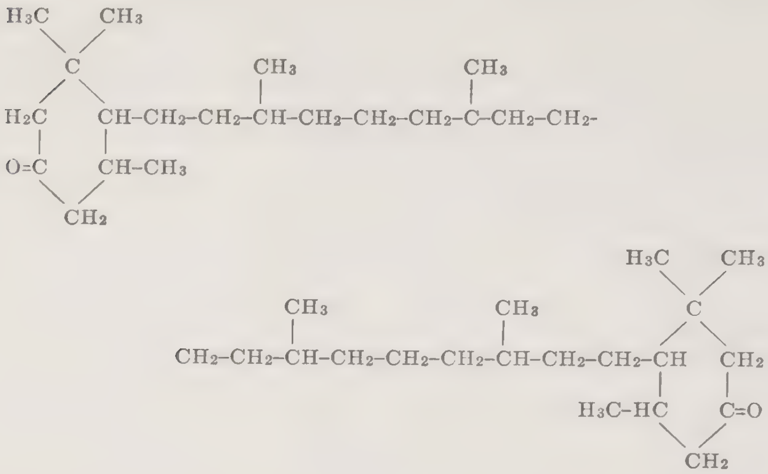
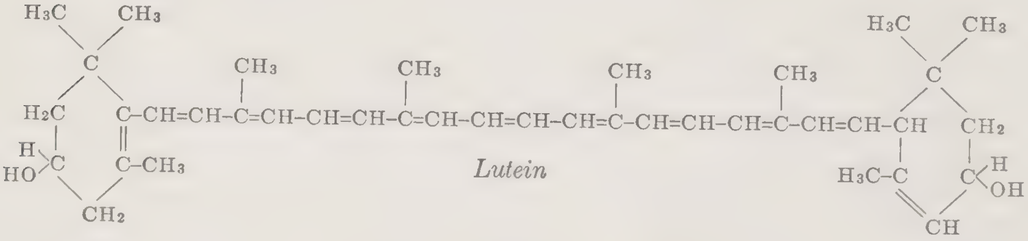
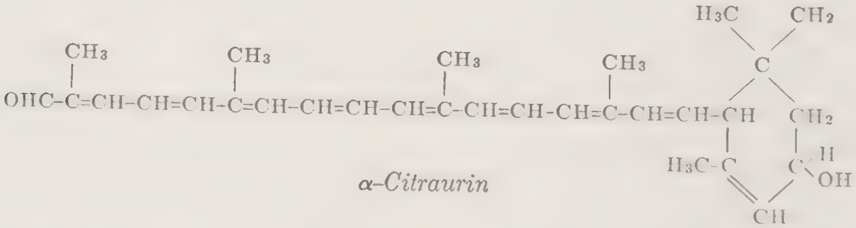


thus confirming the secondary nature of the hydroxyl groups. Lutein is distinguished from its isomeride zeaxanthin (see p. 53) by its melting point and optical rotation, zeaxanthin being inactive. Karrer[238] suggested that the double linkages may allow varying arrangements, so that xanthophyll isomerides may be referred to α- or β-carotene. The absorption spectrum of optically active lutein agrees closely with that of α-carotene and a dihydro-α-carotene structure[239] is suggested:



Lutein

α-Citraurin[240] [$C_{30}H_{40}O_2$, orange-red leaflets, m.p. 153°, (oxime $C_{30}H_{41}O_2N$, m.p. 148°)] is obtained by oxidizing lutein with potassium permanganate:



α-Citraurin

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[238] Nilsson, Karrer: *Ibid.*, 14, 843 (1931).

[239] *See* Karrer, Morf. v. Krauss, Zubrys: *Ibid.*, **15**, 490 (1932). Karrer, Zubrys, Morf: *Ibid.*, 16, 977 (1933).

[240] Karrer, Koenig, Solmssen: *Ibid.*, 21, 445 (1938).

Experiment has shown that highly purified preparations [241] of lutein possess no growth action. Lutein [242] is best obtained from nettle pulp.

The so-called curcubitaxanthin [243] is not a homogeneous compound, but consists of lutein, with a little violaxanthin [244] and is therefore to be deleted from the literature.

An isomeride of lutein [245] [m.p. 205-206° (corr.)] was isolated with other carotenoids from the yellow flowers of the furze (*Ulex europaeus*).

The yellow plumage pigment of canary birds [246] contains a carotenoid with sharp absorption bands at 472-443-418 $m\mu$ (petroleum), which is similar to taraxanthin. As the isolation of this pigment presented difficulty because of lack of material, a strain of canaries with colorless feathers was produced by feeding with material which was free from carotenoids. Addition of lutein to the diet caused the yellow color of the plumage to reappear, only zeaxanthin of all other carotenoid pigments being able to replace it. As lutein is found in the liver, body-fat and egg-yolk of the bird, the plumage pigment (the so-called canary xanthophyll) would seem to be a modified lutein. The plumage of other birds also contains lutein and canary xanthophyll, together with decomposition products imparting a red tint which comprise the so-called *picofulvin* of Krukenberg.

Heleniene.[247] This pigment ($C_{72}H_{116}O_4$, red needles, m.p. 92°), which is the dipalmatic ester of lutein, occurs in *Helenium autumnale*.[248] Preparations from *Tagetes* melt below 92° so that in this flower other fatty acids are probably concerned in the esterification of lutein. The presence of palmitic acid [249] may be genetically connected with the occurrence of hentriacontane, $C_{31}H_{64}$, in *Tagetes* blooms, as evidence is also available that the intermediate palmitone $(C_{15}H_{31})_2CO$ also occurs.

Zeaxanthin [250, 251] [$C_{40}H_{56}O_2$, pale yellow tablets, m.p. 215,5°, $[\alpha] \frac{20}{\mathrm{Cd}} = \pm 5^\circ$ (benzene)[252] is the chief pigment of maize (*Zea mais*) which also contains a small quantity of yellow coloring matter soluble in alkali and probably belonging to the flavone series. Zeaxanthin is oxidized in the air, at first slowly, then more rapidly until the color completely disappears. It is also found in the seed husks of *Evonymus europaeus* (burning bush[253]) in which about 75 per cent of the zeaxanthin is unesterified, in the red Kaki fruit (*Diospyros Kaki*),[254] and as an ester in the flowers of leopard's bane[255] (*Senecio Doronicum*). As already mentioned under lutein, Kuhn[256] has shown that the pigment of egg-yolk consists of a mixture in varying proportions of lutein and zeaxanthin.

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[241] Kuhn, Brockmann: *Z. physiol. Chem.*, **221**, 129 (1933); earlier investigations: B. and H. v. Euler, Karrer: *Helv. Chim. Acta*, **12**, 278 (1929); H. v. Euler, Karrer, Rydbom: *Ibid.*, **13**, 1059 (1930); **14**, 1428 (1930); see further, H. v. Euler, Karrer, Zubrys: *Ibid.*, **17**, 24 (1933).

[242] Willstätter, Mieg: *Ann.*, **355**, 1 (1907); new method of preparation: Willstätter, R., and Stoll, A., "Untersuchungen über Chlorophyll," p. 327; Karrer, Helfenstein, Pieper, Morf: *Helv. Chim. Acta*, **14**, 614 (1931); purification: Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).

[243] Suginome, Ueno: *Bull. Chem. Soc. Japan*, **6**, 221 (1931).

[244] Zechmeister, Tuzson: *Ber.*, **67**, 824 (1934).

[245] Schön: *Biochem. J.*, **30**, 1960 (1936).

[246] Brockmann, Völker: *Z. physiol. Chem.*, **224**, 193 (1934).

[247] Kuhn, Winterstein: *Naturwiss.*, **18**, 754 (1930); Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).

[248] Flowers in which heleniene occurs: Kuhn, Winterstein: *Naturwiss.*, **18**, 754 (1930).

[249] See also Chammon, Chibnall: *Biochem. J.*, **23**, 168 (1929).

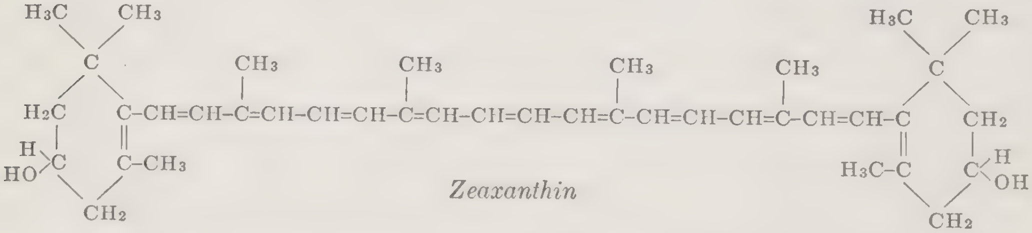
[250] Earlier literature: Thudichum: *Proc. Roy. Soc. London*, **17**, 253 (1869); Palmer, Eckles: *Missouri Agricultural Expt. Sta. Res. Bull.*, **10**, 339 (1914).

[251] Karrer, Salomon, Wehrli: *Helv. Chim. Acta*, **12**, 790 (1929).

[252] Kuhn, Grundmann: *Ber.*, **67**, 596 (1934).

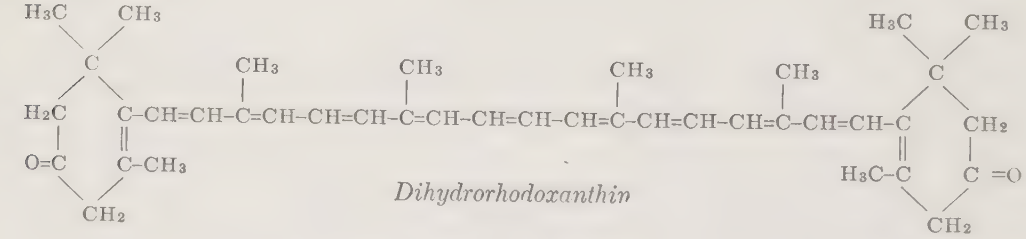
A Zerewitinoff determination shows that the oxygen is present in the form of a hydroxyl group.[257] Zeaxanthin behaves on oxidation[258] with potassium permanganate as does lutein, giving α,α-dimethylsuccinic acid. Ozonolysis yields no acetone, whereas oxidation with chromic acid[259] affords 6 mols. of acetic acid and 27.5 mols. of carbon dioxide. Hydrogenation reveals 11 double linkages, and toluene and *m*-xylene are produced by thermal decomposition.[260] The absorption spectrum of zeaxanthin closely resembles that of β-carotene.

All these observations[261] are in agreement with the following formula for zeaxanthin as a derivative of optically inactive β-carotene:



Zeaxanthin

Further support for this constitution is provided by the conversion[262] of dihydrorhodoxanthin into zeaxanthin by aluminum isopropylate:



Dihydrorhodoxanthin

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[253] Zechmeister, Szilárd: *Z. physiol. Chem.*, **190**, 67 (1930); Zechmeister, Tuzson: *Ibid.*, **196**, 199 (1931).

[254] Karrer, Morf, v. Krauss, Zubrys: *Helv. Chim. Acta*, **15**, 490 (1932).

[255] Karrer, Notthafft: *Ibid.*, **15**, 1195 (1932).

[256] Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).

[257] Karrer, Helfenstein, Wehrli: *Helv. Chim. Acta*, **13**, 87 (1930).

[258] Karrer, Wehrli, Helfenstein: *Ibid.*, **13**, 268 (1930).

[259] Kuhn, Winterstein, Kaufmann: *Ber.*, **63**, 1489 (1930).

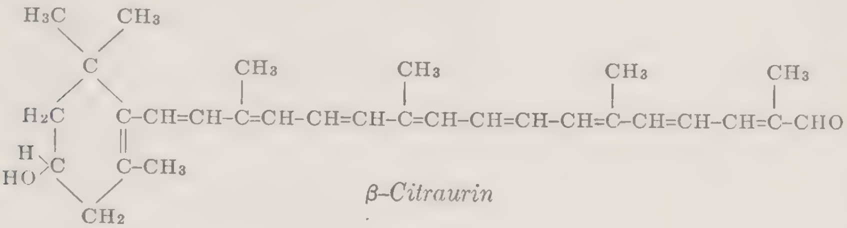
[260] Kuhn, Winterstein: *Ber.*, **65**, 1873 (1932).

[261] Karrer, Morf, v. Krauss, Zubrys: *Helv. Chim. Acta*, **15**, 490 (1932).

[262] Karrer, Solmssen: *Ibid.*, **18**, 477 (1935).

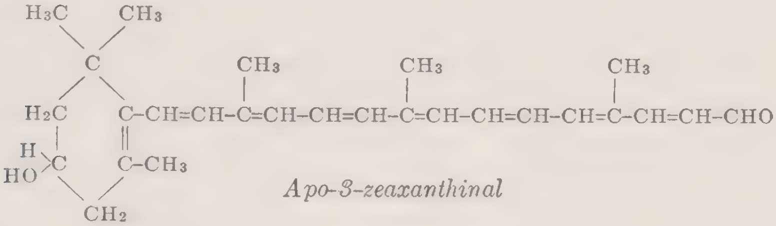
Zeaxanthin is best obtained from *Physalis* calyx[263] by saponification of the physalien (q.v.).

Oxidation of zeaxanthin yields β-citraurin[264] (apo-2-zeaxanthinal $C_{30}H_{40}O_2$, oxime $C_{30}H_{41}O_2N$, m.p. 196°):



β-Citraurin

together with apo-3-zeaxanthinal:



Apo-3-zeaxanthinal

Physalien[265] ($C_{72}H_{116}O_4$, flat prisms or needles, orange-red by transmitted light and fiery-red with a blue reflex by reflected light, m.p. 98.5-99.5°) is the dipalmitic ester of zeaxanthin into which components[266] it may be broken down and thence re-esterified. It was first detected in the winter-cherry (*Physalis Alkekengi*)[267] and *Physalis Franchetti*. The green sepals[267] contain xanthophyll and carotene in the ratio 3:1 as normal concomitants of chlorophyll. If the fading of the sepals is artificially induced by treatment with oxygen, the synthesis of physalien commences, while the carotene content increases considerably and that of xanthophyll decreases. The phytol content[268] of the green sepals, which has also been determined, is insufficient to account for the maximum observed formation of physalien, and the plant either possesses reserves of phytol or the synthesis of physalien proceeds by some other mechanism. Physalien is also found in goat's thorn[269] (*Lycium halimifolium*). On ozonolysis [270] a small amount of azelaic acid was obtained, thus indicating the participation of a further acid, presumably oleic, besides palmitic acid in the production of physalien. Synthetic esters [271] with lauric, myristic and stearic acids which resemble physalien have also been obtained.

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[263] Kuhn, Winterstein, Kaufmann: *Ber.*, **63**, 1489 (1930).

[264] Karrer, Solmssen: *Helv. Chim. Acta*, **20**, 682 (1937); Karrer, Solmssen, Gugelmann: *Ibid.*, **20**, 1020 (1937); Karrer, Rüegger, Solmssen: *Ibid.*, **21**, 448 (1938). Isomerization of zeaxanthin: Zechmeister, v. Cholnoky, Polgár: *Ber.*, **72**, 1678 (1939).

[265] Kuhn, Wiegand: *Helv. Chim. Acta*, **12**, 499 (1929) (earlier literature previous to the characterization of the pigment).

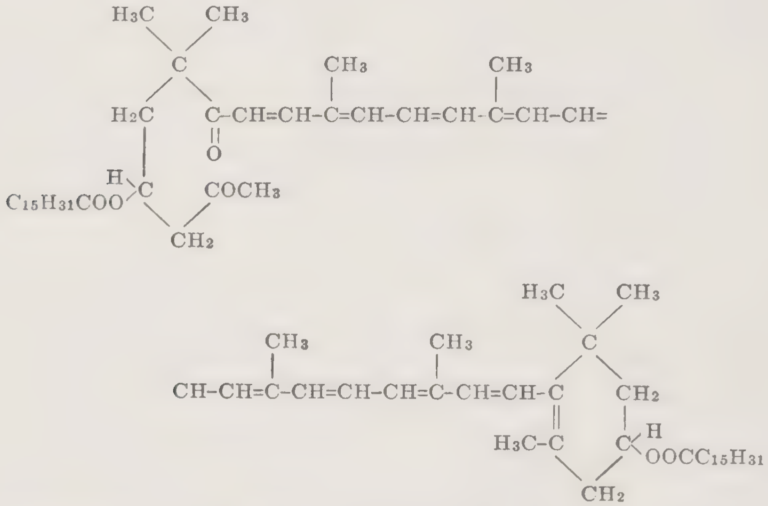
[266] Zechmeister, v. Cholnoky: *Ann.*, **481**, 42 (1930); Kuhn, Winterstein, Kaufmann: *Naturwiss.*, **18**, 418 (1930); *Ber.*, **63**, 1489 (1930).

[267] Kuhn, Brockmann: *Z. physiol. chem.*, **206**, 41 (1932).

[268] *Cf.* the view of Karrer, Helfenstein, Wehrli, Wettstein: *Helv. Chim. Acta*, **13**, 1084 (1930) and p. 1088.

[269] Zechmeister, v. Cholnoky: *Ann.*, **481**, 42 (1930); *Z. physiol. Chem.*, **189**, 159 (1930).

Zeaxanthin monopalmitic ester [272] (m.p. 148°), which is spectroscopically identical with zeaxanthin and with physalien, has been prepared by partial saponification of physalien itself. Among the mixture of products obtained by oxidizing physalien with chromic acid [273] a diketone and a tetraketone were found. The former, which was obtained in a state of purity, formed carmine-red needles, m.p. 144-145°, absorption bands 538-503 mμ (CS_2), and is assigned the constitution:



Pigments of Egg-yolk. The preparation of the pigment of egg-yolk was attempted by Chevreul, Gobley and Städeler.[274] The term *lutein*, originated by Thudichum,[275] has today a somewhat modified significance. Willstätter and Escher [276] obtained a preparation which had a melting point of 195-196° and a composition corresponding to $C_{40}H_{56}O_2$; but it was Kuhn, Winterstein and Lederer [277] who first succeeded in demonstrating that egg-yolk pigment consists of a varying mixture of lutein (m.p. 195°) and zeaxanthin (m.p. 215°). It was possible, by controlling the food of the hens, to obtain egg-yolk in which zeaxanthin occurred almost exclusively, or in which lutein occurred to a predominating extent. The separation of the mixture of pigments was effected by adsorption on a column of calcium carbonate.

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[270] Karrer, Pieper: *Helv. Chim. Acta*, **14**, 838 (1931); Isomerization of physalien: Zechmeister, v. Cholnoky, Polgár: *Ber.*, **72**, 1678 (1939).

[271] Kuhn, Winterstein, Kaufmann: *Ber.*, **63**, 1489 (1930).

[272] Karrer, Schlientz: *Helv. Chim. Acta*, **17**, 55 (1934).

[273] Karrer, Solmssen, Walker: *Helv. Chim. Acta*, **17**, 417 (1934); Karrer, Gugelmann: *Ibid.*, **20**, 405 (1937).

[274] Städeler: *J. prakt. Chem.*, **100**, 148 (1867).

[275] Thudichum: *Proc. Roy. Soc. London*, **17**, 253 (1869); for further work see Willstätter, Escher: *Z. physiol. Chem.*, **76**, 214 (1912); spectrometric results: Schunck: *Proc. Roy. Soc. London*, **72**, 165 (1904).

[276] Willstätter, Escher: *Z. physiol. Chem.*, **76**, 214 (1912); Cf. the work of Serono [*Arch. Farmacol. sper.*, **11**, 553 (1911); **14**, 509 (1914)] which is at the present time valueless; Palmer: "Carotenoids and Related Pigments," p. 139, 176; Barbieri [*Compt. rend.*, **154**, 1726 (1912)] describes a pigment, ovochromin, from egg-yolk.

[277] Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931); spectrophotometric analysis: Kuhn, Smakula: *Z. physiol. Chem.*, **197**, 161 (1931).

Eschscholtz-xanthin[278] ($C_{40}H_{54\pm2}O_2$, purple-red crystals, m.p. 185-186° $[\alpha]\dfrac{18}{\mathrm{Cd}} = +225° \pm 12°$) is found in the petals of the California poppy, *Eschscholtzia california*. It contains 12 conjugated double bonds and 2 hydroxyl groups which may be esterified.

Flavoxanthin[279] [$C_{40}H_{56}O_3$, thick salmon-red or golden-yellow prisms, m.p. 184°, $[\alpha]\dfrac{20}{\mathrm{Cd}} = +190°$ (benzene); absorption bands 450-422 mμ (petroleum, b.p. 70-80°)] occurs in the petals of the buttercup, (*Ranunculus acer*) and has also been detected in groundsel (*Senecio vernalis*). It was formerly termed *flavoxanthin,* as it is the most yellow of all known xanthophylls, in marked contrast to the red rhodoxanthin. In the above flowers four different xanthophylls are known to occur, about 50 per cent existing in an esterified form. Predominating is lutein, then a pigment, which is distinguished by the strong blue color given by hydrochloric acid and which is probably identical with Tswett's xanthophyll-β; and taraxanthin, probably violaxanthin and finally flavoxanthin are also present. Purification is effected by partitioning between petroleum and methanol, and they may be separated also by chromatographic analysis using calcium carbonate.

Flavoxanthin takes up 11 mols. of hydrogen; two carbocyclic rings are therefore present. Zerewitinoff determination reveals that all three oxygen atoms are present as hydroxyl groups. The presence of three hydroxyl groups is reflected in the absorption behavior and in its behavior on partitioning between petroleum and methanol. The extremely short wave-lengths at the absorption band-heads suggest that all the double bonds may not be conjugated. On introducing 25-per cent hydrochloric acid beneath an ethereal solution of flavoxanthin, a deep blue coloration is produced, which turns violet and fades within 5-10 minutes (distinction from violaxanthin). One kg. of dried leaves yields 40 mg. of pure pigment.

Antheraxanthin[280] [$C_{40}H_{58(-2?)}O_3$, leaflets, m.p. 211°, absorption bands 512.5-481-448 mμ (CS_2)] occurs in the tiger lily (*Lilium tigrinum*) accompanied by capsanthin; it possibly occurs also in the cranberry.[281] Antheraxanthin bears a resemblance to zeaxanthin.

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[278] Strain: *J. Biol. Chem.*, 123, 425 (1938).

[279] Kuhn, Brockmann: *Z. physiol. Chem.*, 213, 192 (1932); for earlier examination of *Ranunculus* species, see Escher: *Helv. Chim. Acta,* 11, 752 (1928), who obtained from *R. Steveni Andrz.* a crystalline xanthophyll; Karrer, Notthafft: *Ibid.*, 15, 1195 (1932) also obtained a xanthophyll (m.p. 185°) from *R. arvenis,* the absorption bands of which are identical with those of lutein.

[280] Karrer, Oswald: *Helv. Chim. Acta,* 18, 1303 (1935); Willstaedt: *Chem. Zentr.*, 1937, I, 2620.

Petaloxanthin [281] [$C_{40}H_{58(-2?)}O_3$, sharp needles, m.p. 211-212° (corr.), absorption bands 514.5-481 mμ (CS_2)] is found in the fading calyx of the marrow plant with other carotenoids. It is very similar to but not identical with antheraxanthin.

Eloxanthin [282] [$C_{40}H_{56}O_3$, glistening bronze leaflets, m.p. 182.5-183°, absorption bands 502-472-444 mμ (CS_2)] occurs in pond-weed (*Elodea canadensis*). It contains 11 double linkages, of which 9 are conjugated; it also contains 3 hydroxyl groups; eloxanthin is optically active $[\alpha]_D^{18} = +225°$ (benzene).

Violaxanthin [283] [$C_{40}H_{56}O_4$, reddish-brown needles or yellow-brown prisms, m.p.[284] 207-208°, $[\alpha]\frac{20}{\mathrm{Cd}} = +35°$ (chloroform), absorption bands 472-443 mμ (petroleum, b.p. 70-80°)] was first found in the petals of the yellow pansy (*Viola tricolor*) where it is accompanied by quercetrin. It is possibly identical with Schunck's [285] xanthophyll Y and would seem to be contained in Tswett's [286] xanthophyll α' and α''. It probably occurs in the yellow orange and the mandarine orange [287] and has also been detected in the cocoon of the Japanese silk-worm,[288] in *Calendula officinalis* (marigold),[289] in *Tragopogon pratensis, Laburnum, Sinapis officinalis* [290] and finally in leaves, *e.g.*, of the horse-chestnut.[291] Violaxanthin exists in the petals of the yellow pansy in the form of an ester which has not yet been obtained in a state of purity; it is not impossible that the pigment exists simultaneously in varying stages of esterification, and certainly saponification yields a mixture of solid fatty acids.

The color reactions with dilute mineral acids are striking and recall those of fucoxanthin. If an ethereal solution of violaxanthin is shaken with 47-per cent sulfuric acid (the color reaction may also be observed using 20.5-25-per cent hydrochloric acid) the aqueous layer assumes a blue color; these blue salts seem to be soluble only in ethereal sulfuric acid.

On partitioning between methanol and petroleum ether, violaxanthin takes up a position intermediate between lutein and fucoxanthin, remaining under controlled conditions to a major extent in the lower layer. From the results of Zerewitinoff determinations carried out on perhydroviolaxanthin ($C_{40}H_{78}O_4$), Kuhn[292] concluded that all of the four oxygen atoms are present as hydroxyl groups. Karrer,[293] however, could find only three hydroxyl groups, both in the perhydroderivative and in the parent violaxanthin which contains ten ethylenic bonds.[294]

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[281] Zechmeister, Béres, Ujhelyi: *Ber.*, **69**, 573 (1936).
[282] Hey: *Biochem. J.*, **31**, 532 (1937).
[283] Kuhn, Winterstein: *Ber.*, **64**, 326 (1931).
[284] Karrer, Morf: *Helv. Chim. Acta*, **14**, 1044 (1931).
[285] Schunck: *Proc. Roy. Soc. London*, **72**, 165 (1904).
[286] *See* Kuhn, Brockmann: *Z. physiol. Chem.*, **206**, 41 (1932), and p. 60.
[287] Zechmeister, Tuzson: *Naturwiss.*, **19**, 307 (1931).
[288] Oku: *Bull. Agr. Chem. Soc. Japan*, **9**, 91 (1933).
[289] Zechmeister, v. Cholnoky: *Z. physiol. Chem.*, **208**, 26 (1932).
[290] Karrer, Notthafft: *Helv. Chim. Acta*, **15**, 1195 (1932).
[291] Kuhn, Winterstein, Lederer: *Z. physiol. Chem.*, **197**, 141 (1931).

Violaxanthin gives α,α-dimethylsuccinic acid on oxidation with potassium permanganate[294] behaving in this respect like α-carotene, and probably containing therefore the same ring system. It has not yet been possible to decide whether the hydroxyl groups are attached to adjacent carbon atoms.[295] Perhydroviolaxanthin is not attacked by lead tetracetate which is a specific reagent for α-glycols.

Besides violaxanthin another pigment, which has not yet been obtained in crystalline form and which is extracted from ethereal solution even by 3-per cent hydrochloric acid with production of a blue color, is concentrated in the mother liquor. It is present to the extent of about 20 per cent of the violaxanthin and possesses absorption bands at 496-465-436.5 mμ (CS_2). The dried petals yield 0.05-0.07 per cent pure violaxanthin.

Taraxanthin[296] [$C_{40}H_{56}O_4$, shining ochre-red prisms, m.p. 185.5°, $[\alpha]\frac{20}{\mathrm{Cd}} = +200°$ (ethyl acetate), absorption bands 472-443 mμ (petroleum, b.p. 70-80°)] was first isolated in the pure state from the dandelion (*Taraxacum officinale*) in the form of an ester, where it is accompanied by lutein, probably by violaxanthin and by xanthophyll esters. The three carotenoids may be separated only by chromatographic analysis. Taraxanthin occurs also in colt's foot (*Tussilago farfara*),[297] in the flowers of touch-me-not (*Impatiens noli me tangere*), where it exists almost exclusively in the esterified form, in *Leontodon autumnale*,[298] and finally in the buttercup.[299]

Taraxanthin takes up 10.65 mols. of hydrogen on catalytic reduction and the Zerewitinoff determination corresponds to 3.25 active hydrogen atoms. It gives no color reaction with 25-per cent hydrochloric acid. The isomerism existing between taraxanthin and violaxanthin must be of a different type from that relating lutein to zeaxanthin. The latter present marked differences in their absorption spectra, indicating differences in the systems of conjugated double linkages, whereas the former with almost identical spectra must owe their isomerism to different orientations of the hydroxyl groups. Thirty grams of the bloom of touch-me-not yield almost four mg. of pure taraxanthin.

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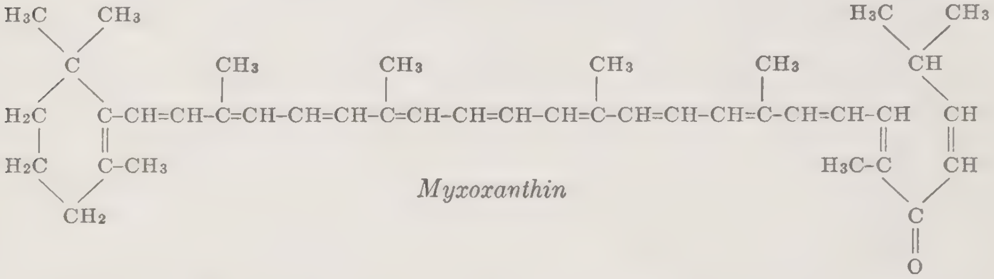
[292] Kuhn, Winterstein: *Ber.*, **64**, 326 (1931).
[293] Karrer, Morf: *Helv. Chim. Acta*, **14**, 1044 (1931).
[294] Karrer, Solmssen: *Ibid.*, **19**, 1024 (1936).
[295] Karrer, Zubrys, Morf: *Ibid.*, **16**, 977 (1933).
[296] Kuhn, Lederer: *Z. physiol. Chem.*, **200**, 108 (1931); see also Schunck: *Proc. Roy. Soc. London*, **72**, 165 (1904), and Karrer, Salomon: *Helv. Chim. Acta*, **13**, 1063 (1930).
[297] Karrer, Morf: *Helv. Chim. Acta*, **15**, 863 (1932).
[298] Kuhn, Lederer: *Z. physiol. Chem.*, **213**, 188 (1932).
[299] Kuhn, Brockmann: *Ibid.*, **213**, 192 (1932).

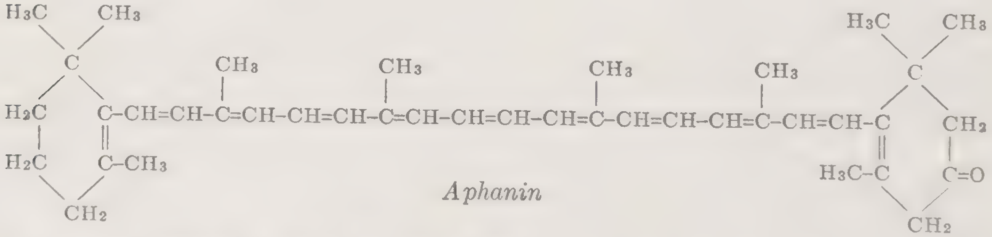
Myxoxanthin[300] [$C_{40}H_{54}O$, deep-violet prisms, m.p. 168-169°, absorption bands 488 mμ (CS_2)] is found in fresh-water algae (*Myxophyceae, e.g., Oscillatoria rubrescens,* accompanied by β-carotene, lutein and myxoxanthophyll. From the results of reduction, which leads to an alcohol, myxoxanthol (spectroscopically identical with γ-carotene) the formation of an oxime, from the absorption spectrum, and the fact that the pigment possesses growth activity indicating an unsubstituted ionone ring, the following constitution is suggested:



Myxoxanthin

Echinenone[301] [$C_{40}H_{58\pm2}O$, glistening violet needles, m.p. 192-193°, absorption bands (not sharp) 520-488-450 mμ (CS_2)] is isolated together with α- and β-carotene by extracting the ova of the sea-urchin (*Strongylocentrotus lividus*) with acetone, transferring the pigments to petroleum ether and then saponifying. It is probably a monoketone intermediate between β-carotenone and semi-β-carotenone. It has no acid character, possesses vitamin activity and in other ways resembles myxoanthin.

Aphanin[302] [$C_{40}H_{54}O$, blue-black leaflets, m.p. 180°, absorption bands 533.5-494 mμ. (CS_2) is an epiphasic pigment found in the blue alga *Asphanizomenon flos-aquae* together with carotene, aphanicin, flavazin and aphanizophyll. It possesses 12 double bonds and one carbonyl group; the latter is not in conjugation with the double bonds. Aphanin shows vitamin A activity, but only one-half of that of β-carotene. The following formula is proposed:



Aphanin

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[300] Heilbron, Lythgoe: *J. Chem. Soc.*, **1936**, 1376.
[301] Lederer: *Compt. rend.*, **201**, 300 (1935); *Bull. soc. Chim. Biol.*, **20**, 567 (1938).
[302] Tischer: *Z. physiol. Chem.*, **251**, 109 (1938); **260**, 257 (1939).

Rhodoxanthin [$C_{40}H_{50}O_2$, blue-black, highly glistening leaflets, m.p. 219°, absorption bands 524-489-458 mμ (petroleum, b.p. 70-80°], previous to its isolation in a state of purity from the fleshy calyx (arillus) of the ripe seed of the yew (*Taxus baccata*),[303] was first observed by Monteverde[304] in the leaves of *Potamogeton natans,* and by Tswett[305] in the leaves of the "tree of life" (*Thuja orientalis*) in *Cupressus Naitnocki, Retinospera plumosa, Juniperus virginica* and *Taxus baccata.* Monteverde and Lubimenko[306] (Lipmaa[307] also examined the pigment) obtained a crystalline product, giving it its present name, although it also appears in the literature as "red xanthophyll."[308] They found the same pigment in the leaves of *Selaginella* and *Gnetum,* as well as in *Taxus.*

Rhodoxanthin, for determining the constitution of which Kuhn and Brockmann[309] were responsible, gives bluish-red solutions in carbon disulfide and contains no methoxyl groups; the low proportion of hydrogen is in agreement with the unusually large number of conjugated double bonds, which in turn may be correlated with the positions of the absorption bands toward the long wave-lengths. Kuhn and Brockmann were unable to obtain the alkali salts mentioned by Lipmaa. The Zerewitinoff determination is in agreement with the presence of one hydroxyl group, but the real nature of both oxygen atoms is disclosed by the formation of a dioxime, $C_{40}H_{52}O_2N_2$, revealing two carbonyl groups. On catalytic hydrogenation, rhodoxanthin first takes up 12 mols. of hydrogen rapidly, then 2 mols. more slowly, absorbing 14 mols. in all. As the reduction of carbonyl groups to hydroxyls is known to take place more slowly, it may be concluded that rhodoxanthin contains 12 double linkages in addition to the two carbonyl groups, and hence two carbocyclic rings must also be present. Reduction with zinc dust affords a dihydro-compound, $C_{40}H_{52}O_2$, with the same melting point, 219°, as rhodoxanthin, which can absorb a further 13 mols. of hydrogen. A lightening in color is associated with this uptake of two hydrogen atoms, just as occurs on converting bixin to dihydrobixin. Dihydrorhodoxanthin, which may easily be confused optically with β-carotene or zeaxanthin, gives a dioxime which is optically identical with the parent dihydrorhodoxanthin, so that manifestly reduction to the dihydro stage has destroyed the conjugation between the carbonyl groups and the other part of the absorbing system. Again, a petroleum solution of rhodoxanthin is orange-yellow in color whereas in alcohol a pure red solution results. This characteristic is lacking in dihydrorhodoxanthin, but is observed with natural carotenoid carboxylic acids, and also with β-carotenone and lycopenal; it must be ascribed on the one hand to the conjugation existing between the polyene chain and the carbonyl groups, and on the other to interaction between the polar carbonyl groups and the polar alcohol molecules.

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[303] Kuhn, Brockmann: *Ber.*, **66**, 828 (1933).

[304] Monteverde: *Acta Horti Petropol.*, 13, 121 (1893); see also Prat, *Biochem. Z.*, 152, 495 (1924).

[305] Tswett: *Compt. rend.*, **152**, 788 (1911).

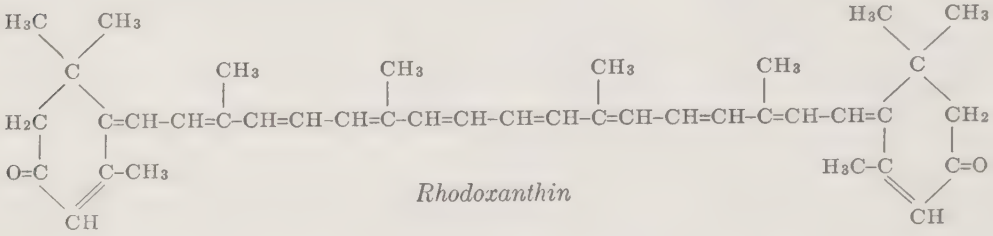
[306] Monteverde, Lubimenko: *Bull. Acad. Sci. Petrograd (6)*, **7**, 1105 (1913).

[307] Lipmaa: *Compt. rend.*, **182**, 867 (1926).

[308] Palmer: "Carotinoids and Related Pigments," p. 216.

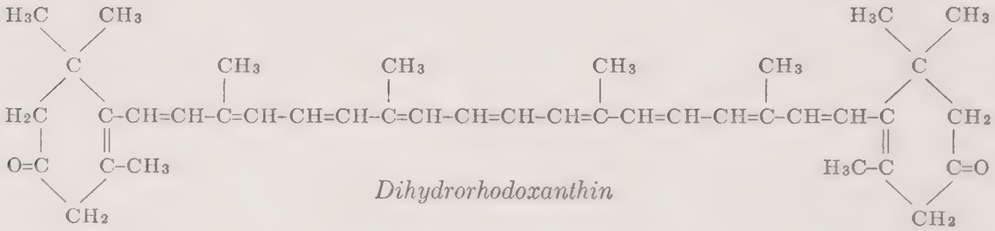
[309] Kuhn, Brockmann: *Ber.*, **66**, 828 (1933).

Dihydrorhodoxanthin forms a violet alkali compound [310] which is oxidized by oxygen to red rhodoxanthin. Taking into consideration all these observations, the following formula is assigned to rhodoxanthin:



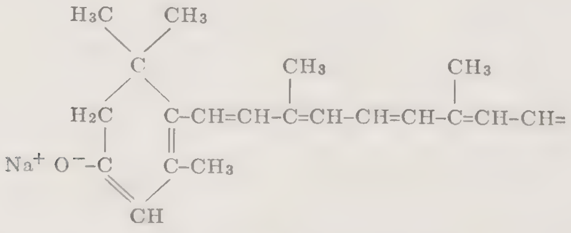
Rhodoxanthin

The pigment is thus symmetrically constituted and both it and its dihydroderivatives are optically inactive. It yields, like β-carotene, 5.5 mols. of acetic acid on oxidation with chromic acid. The evolution of methane in the Zerewitinoff determination may be explained by the proximity of the methylene and ketone groups, although enolization of the CO—CH_2— groups is not effected by alkali. Dihydrorhodoxanthin is given the formula:



Dihydrorhodoxanthin

in which conjugation between the polyene chain and the carbonyl groups has been broken. The alkali compound is thus the dienolate:



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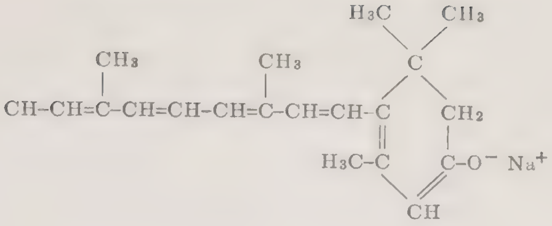
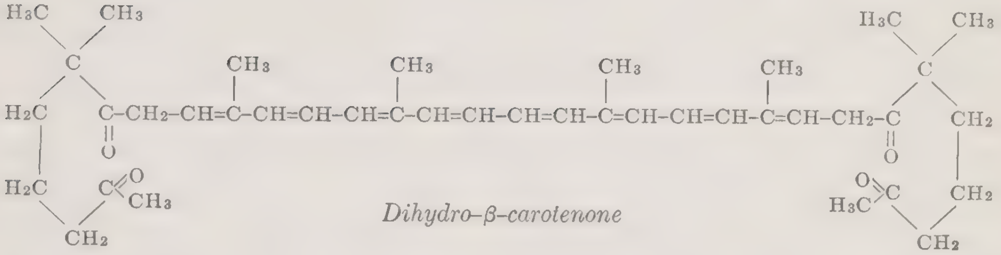
[310] *Cf.* Kuhn, Drumm, Hoffer: Ber., **65**, 1785 (1932), the color reaction of hydropolyene carboxylic esters and also the autoxidation of yellow dihydro-β-carotenone to red β-carotenone.





Dihydro–β–carotenone

The doubly negative charged ion is oxidized by oxygen and the whole system of double bonds is shifted. The identity of the spectra of dihydrorhodoxanthin and zeaxanthin may be correlated with their having the same number and disposition of double linkages. Moreover, just as the close analogy between rhodoxanthin and β-carotenone is explained by their both possessing a chain of 9 ethylenic linkages terminated at each end by carbonyl groups, so the same molecular architecture accounts for the similarity of dihydrorhodoxanthin to dihydro-β-carotene.

The simultaneous occurrence of lycopene, β-carotene and zeaxanthin with rhodoxanthin in the yew lends credence to the view that the hydroxyl groups of zeaxanthin may undergo dehydrogenation, and the resulting dihydrorhodoxanthin may then be further dehydrogenated, as it has been effected *in vitro*. Ten kg. (about 21,000) of the ripe fruits of the yew yield about 65 mg. of almost pure rhodoxanthin.

Capsanthin.[311] The skin of the ripe fruit of Spanish pepper (*Capsicum annuum*), one of the *Solanaceae* largely grown in Hungary and Spain, contains capsanthin as an important constituent in the form of a xanthophyll ester. With the aid of chromatographic analysis [312] the mixture of pigments was completely resolved, the following constituents being recognized:

Polyene alcohols	Acids	Polyene hydrocarbons
Capsanthin	Myristic	α-Carotene
Capsorubin	Palmitic	β-Carotene (traces)
Zeaxanthin	Stearic	
Lutein	Carnaubic	
Kryptoxanthin	Oleic	
Unknown polyenes	Unknown acids	

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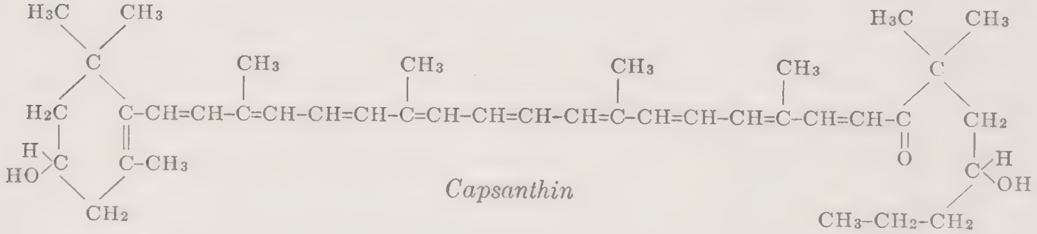
[311] Zechmeister, v. Cholnoky: *Ann.*, **454,** 54 (1927); **455,** 70 (1927); **465,** 288 (1928); **478,** (1930); **487,** 197 (1931); **489,** 1 (1931); Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta*, **14,** 614 (1931).

[312] Zechmeister, v. Cholnoky: *Ann.*, **509,** 269 (1934); see also v. Cholnoky: *Chem. Zentr.*, **1933,** II, 2838.

The ripe pods, "chillies,"[313] of *Capsicum frustescens japonicum*[314] and also *Perfection pimento,*[315] a variety of *Capsicum annuum,* contain capsanthin. Previous workers[316] have examined the pigment. The pigment [$C_{40}H_{58}O_3$, m.p. 175-176° (corr.), absorption bands 505-475 mμ (petroleum, b.p. 70-80°), $[\alpha]_{Cd} = +36°$ (chloroform)], forms dark carmine-red glistening needles which dissolve in alcohol to give a dark-red and in petroleum an orange-yellow solution. It is slowly oxidized in the air, and 25-30-per cent hydrochloric acid produces a greenish coloration. Reduction[317] with aluminum isopropoxide yields capsanthol, $C_{40}H_{57}(OH)_3$, which can take up 10.2 mols of hydrogen. Zechmeister[318] reported that hydrogenation of capsanthin itself reveals the presence of ten double bonds, while two of the oxygen atoms are contained in hydroxyl groups (Zerewitinoff determination and esterification) and the third is present as a ketone group, although the last is so far only evidenced by the reduction of a perhydroproduct, $C_{40}H_{78}O_3$, after which three acetyl residues may be introduced. Capsanthin is thus a dihydroxyketone. The inertness of the carbonyl group as well as the varying color in alcohol and in petroleum is presumably due to an ethylenic linkage in an immediately adjacent position.[319]

Oxidation with potassium permanganate yields α,α-dimethylsuccinic acid and dimethylmalonic acid, and *m*-xylene is obtained by thermal degradation. The following formula probably represents, therefore, the structure of capsanthin:



Capsanthin

It will be observed that this formulation and essentially similar ones are also in agreement with the inactivity of the pigment as a provitamin and with the absence of geronic acid and acetone among the products of oxidation and ozonolysis. Oxidation of capsanthin diacetate yields the diacetate of capsanthinone [$C_{40}H_{56}O_3(OCOCH_3)_2$, prisms, m.p. 123-

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[313] Karrer, Schlientz: *Helv. Chim. Acta,* **17,** 7 (1934).

[314] Zechmeister, v. Cholnoky: *Ann.,* **489,** 1 (1931) contradict the contrary statements of Bilger: *Bull. Basic. Sci. Research:* **3,** 37 (1931).

[315] Brown: *J. Biol. Chem.,* **110,** 91 (1935).

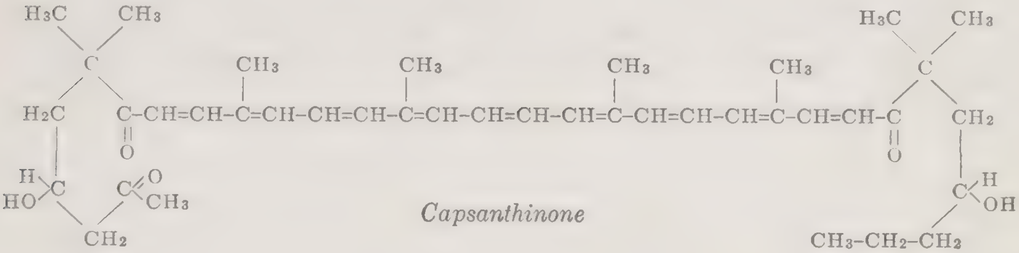
[316] Literature: Zechmeister in Klein, "Handbuch der Pflanzenanalyse," **III,** 2, p. 1318, or Zechmeister: "Carotenoide," p. 237.

[317] Karrer, Hübner: *Helv. Chim. Acta,* **19,** 474 (1936).

[318] Zechmeister: "Carotenoide," p. 237. Zechmeister, v. Cholnoky: *Ann.,* **516,** 30 (1935); **523,** 101 (1936).

[319] *Cf.* Kuhn, Brockmann: *Ber.,* **65,** 894 (1932); **66,** 1319 (1933).

124°]. Capsanthinone, $C_{40}H_{58}O_5$, contains two hydroxyl and three carbonyl groups:



Capsanthinone

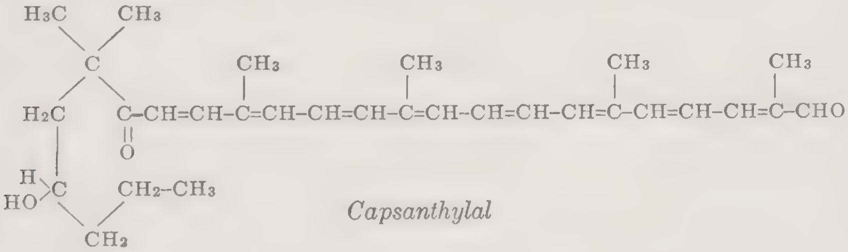
This structure contains two carbonyl groups in 1:6 positions and accordingly loses water in the presence of alkali by cyclization (*cf.* the formation of anhydrosemi-β-carotenone from semi-β-carotenone).

The pigment may be further degraded beyond capsanthinone to yield:

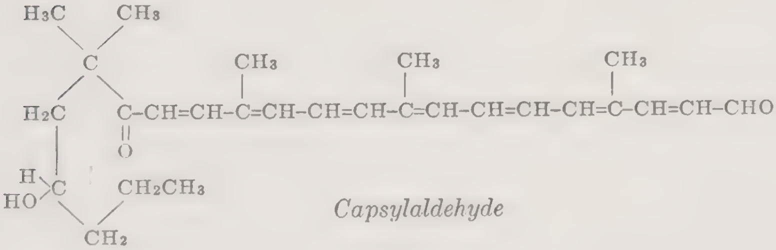
(1) Capsanthylal, $C_{30}H_{42}O_3$, needles, m.p. 127°; oxime, needles, m.p. 184°.

(2) Capsyl aldehyde, $C_{27}H_{38}O_3$; oxime, needles, m.p. 172°.

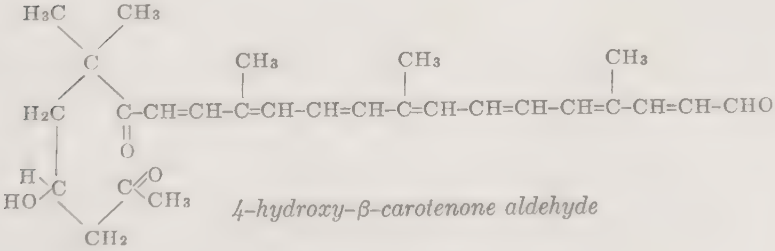
(3) 4-Hydroxy-β-carotenone aldehyde, $C_{27}H_{36}O_4$ oxime, m.p. 189°. For these compounds the following constitutions are probable:



Capsanthylal

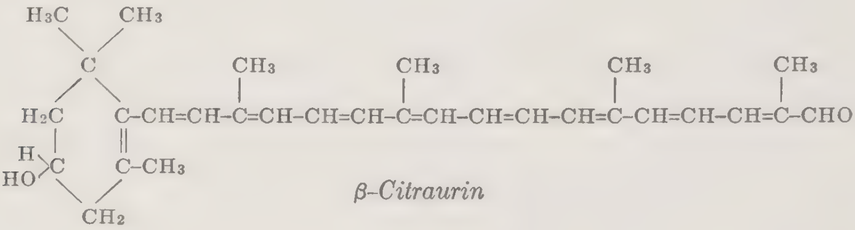


Capsylaldehyde



4-hydroxy-β-carotenone aldehyde

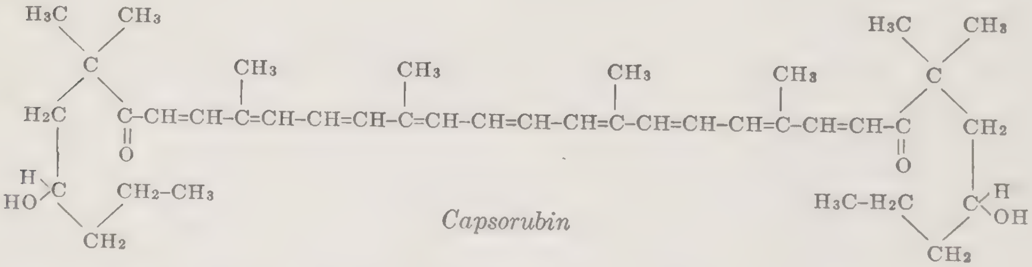
Degradation with a restricted amount of alcoholic potash yields a compound [320] $C_{30}H_{40}O_2$, which proves to be identical with β-citraurin. Its constitution [320] follows from its empirical formula, from the ready formation of an oxime (indicating an aldehyde group) and from the presence of an esterifiable hydroxyl group; it clearly contains the terminal ring of capsanthin unchanged:



β-Citraurin

Citraurin,[322] termed β-Citraurin to distinguish it from the α-carotenoid [cherry-red aggregates of needles, m.p. 144-145°, absorption bands 523-488-457 mμ (CS_2), oxime, m.p. 181-182°] had previously been found in the orange in which it forms the chief pigment together with kryptoxanthin, zeaxanthin, lutein, and violaxanthin, and in which it is present as a complex ester. It is also obtained by oxidizing zeaxanthin acetate.[323]

Capsorubin [324] [$C_{40}H_{60}O_4$, violet-red needles, m.p. 201° (corr.), absorption bands 506-474-444 mμ (petroleum)] is also present in paprika. Capsorubin diacetate forms four-sided leaves [m.p. 179° (corr.)]. The parent capsorubin is a dihydroxydiketone, and spectroscopic evidence indicates that the two ketone groups are associated with the 9 ethylenic linkages to form the whole chromophoric system. The following formula is therefore assigned by analogy with capsanthin:



Capsorubin

Astaxanthin, the coloring matter of the lobster, has been frequently examined and also appears in the literature under the names crustaceorubin, zoönerythrin, vitellorubin, and tetronerythrin,[325] but to Kuhn must be given the credit for first clarifying its chemistry.

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[320] Zechmeister, v. Cholnoky: *Ann.*, 530, 291 (1937).

[322] Zechmeister, Tuzson: *Ber.*, 69, 1878 (1936); 70, 1966 (1937).

[323] Karrer, Solmssen: *Helv. Chim. Acta*, 20, 682 (1937).

[324] Zechmeister, v. Cholnoky [*Ann.*, 516, 30 (1935)] mention without further description another pigment, p. 41, note 3.

The green chromoproteid which occurs abundantly in the egg of the lobster (*Astacus gammarus*) is broken down by alcohol, acetone, dilute acid or by heat treatment when the color changes to red. The red pigment which may be also isolated from the crab[326] and which was previously termed an "ovoester" was later found to be not an ester but a carotenoid—astaxanthin.[327] Of the coloring matters described by Maly[328] vitellorubin is to be identified with astaxanthin and vitellolutein behaves optically and chromatographically like β-carotene. Astaxanthin passes by oxidation with the loss of four hydrogen atoms into astacin, which was originally regarded as the primary pigment of the lobster. It may be that astacin isolated from other *Crustaceae* is obtained only by virtue of the astaxanthin originally present. Immediately below, however, the chemistry of astacin is considered, because, although it is a reaction product of astaxanthin, its examination provides the basis on which the constitution of astaxanthin is founded. Astacin[329] has been isolated from spring lobster (*Palinurus vulgaris*), *Sebastus marinus, Cancer pagurus, Pesca fluvialis, Leander serratus, Portunus puber, Nephrobs, Cyclopterus lumpus,*[330] *Axinella crystagalli, Echinaster sepositus,*[331] *Beryx decadactylus* (goldfish) and *Carassius auriatus* (redfish).[332] These pigments obtained from varieties of fish previously were termed rhodophan, zoönerythron or tetronerythrin.

Astacin occurs also in the pigment of the red papillae around the eyes of the pheasant[333] and a derivative is found in *Astervidea* varieties[334] *e.g.*, in the star fish *Ophidiaster phidianus.* Astacin forms violet needles (m.p. 240-243°, depending on the rate of heating), and possesses a composition[335] corresponding to $C_{40}H_{48}O_4$, which differs from older formulas; the absorption band is 500 mμ (pyridine). Kuhn's work[336] has shown that astacin is stable toward atmospheric oxygen; the ethereal solution shows no color reaction with hydrochloric acid and the pigment possesses no growth activity. A sodium salt and an acetyl derivative were obtained, and although astacin does not react with diazomethane, methylation was slowly effected by dimethylsulfate. Oxidation with chromic acid gave 5 mols. of acetic acid. Karrer[337] demonstrated that on hydrogenation 13 mols. of hydrogen are absorbed and that degradation with potassium permanganate yields dimethylmalonic acid.

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[325] Literature: Kuhn, Lederer: *Ber.*, **66**, 488 (1933).

[326] Kuhn, Lederer, Deutsch: *Z. physiol. Chem.*, **220**, 229 (1933).

[327] Kuhn, Sörensen: *Angew. Chemie*, **51**, 465 (1938); *Ber.*, **71**, 1879 (1938).

[328] Maly: *Monatsh.*, **2**, 351 (1881).

[329] Fabre, Lederer: *Compt. rend. soc. biol.*, **113**, 344 (1933); *Bull. soc. chim. biol.*, **16**, 105 (1934); Willstaedt: *Svensk. Kem. Tid.*, **46**, 205, 261 (1934).

[330] Sörensen: *Z. physiol. Chem.*, **235**, 8 (1935); *Chem. Zentr.*, **1935**, I, 2551.

[331] Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 915 (1935).

[332] Lederer: *Compt. rend. soc. biol.*, **118**, 542 (1935); *Bull. soc. chim. biol.*, **20**, 554 (1938). No astacin was found in *Actinia equina, Antedon rosacea* and *Suberites domuncala*: Kuhn, Lederer, Deutsch: *Z. physiol. Chem.*, **220**, 229 (1933).

[333] Brockmann, Völker: *Z. physiol. Chem.*, **224**, 193 (1934).

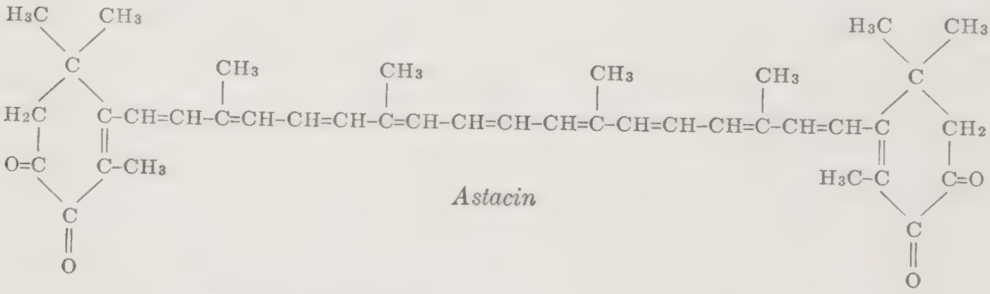
[334] Karrer, Benz: *Helv. Chim. Acta*, **17**, 412 (1934). Review on the occurrence of astaxanthin: Kuhn, Stene, Sörensen: *Ber.*, **72**, 1688 (1939).

[335] Karrer, Loewe: *Helv. Chim. Acta*, **17**, 745 (1934).

[336] Kuhn, Lederer, Deutsch: *Z. physiol. Chem.*, **220**, 229 (1933).

In astacin two carbonyl groups are reactive toward hydroxylamine, giving a dioxime, $C_{40}H_{50}O_4N_2$; and two more undergo enolization in the presence of alkali, so that the dioxime contains four active hydrogen atoms in the sense of the Zerewitinoff determination (two enolic and two oxime hydrogen atoms). Astacin itself, on the other hand, evolves very little methane on treating with Grignard reagent and is thus enolized to only a very small extent (*cf.* the behavior toward diazomethane). Of the 13 double linkages, two are assumed to be due to enolization; in agreement with this conception perhydroastacin contains, according to the Zerewithinoff determination, two hydroxyl groups.

Compared with carotene, astacin contains four carbonyl oxygen atoms in place of eight hydrogen atoms; and, as it forms a diphenazine derivative, it must contain two pairs of carbonyl groups in the 1:2 position, Reduction experiments[338] and the oxidative degradation[339] of the diphenazine compound support the constitution:



Astacin

Astaxanthin,[340] $C_{40}H_{52}O_4$, forms glistening tablets, which melt at 215-216° (decomp.). Among esters of astaxanthin, the diacetate forms deep blue-black needles (m.p. 203-205°), the dicaprylate, dark red granules (m.p. 121-124°), and the dipalmitate, violet-red needles, (m.p. 71.2-72.5°). Oxidation with two atoms of oxygen converts astaxanthin into astacin, the four keto-groups of the latter originating as two ketone and two secondary hydroxyl groups. Two active hydrogen atoms may therefore be detected. As it seems probable that the carbonyl groups are conjugated with the polyene chain, astaxanthin is formulated as 5-5′dihydroxy-4-4′-diketo-β-carotene:

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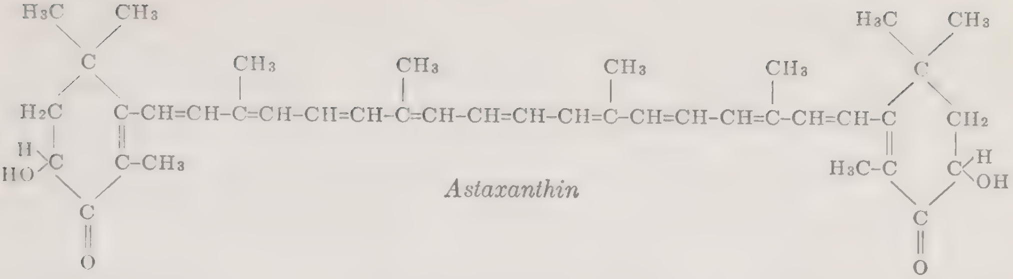
[337] Karrer, Benz: *Helv. Chim. Acta*, **17**, 412 (1934); Karrer, Loewe: *Ibid.*, **17**, 745 (1934).

[338] Willstaedt: *Svensk. Kem. Tid.*, **46**, 205 (1934).

[339] Karrer, Loewe, Hübner: *Helv. Chim. Acta*, **18**, 96 (1935).

[340] Kuhn, Sörensen: *Ber.*, **71**, 1879 (1938); for earlier work on the esters see Karrer, Hübner: *Helv. Chim. Acta*, **19**, 479 (1936); Karrer, Loewe, Hübner: *Ibid.*, **18**, 96 (1935). Astaxanthin seems to exist in the free state in the trout: Sörensen, Stene: *Chem. Zentr.*, **1939**, I, 3749.



Astaxanthin

In complete absence of oxygen, astaxanthin gives deep blue alkali salts but on admission of air, dehydrogenation to astacin takes place. The molecular ratio of astaxanthin to its specific protein is 1:242, from which the molecular weight of ovoverdin is about 144,000. The epiphasic pigments found in the epidermis of the lobster are esters of astaxanthin. 2.5 kg. of lobster ova yielded 0.75 gram of astaxanthin.

A pigment closely related to astaxanthin has been found in *Pleuroplauctus elegans.*[341]

Fucoxanthin is found in the brown algae[342] (*Phycophyceae*), the term *fucoxanthin* being preferred by Sorby[343] to that of Millardet[344] (phylloxanthin). Closer examination by Willstätter and Page[345] led them to assign the formula $C_{40}H_{54}O_6$, later modified by Karrer[346] to $C_{40}H_{56}O_6$, while Heilbron[347] regards $C_{40}H_{60}O_6$ as correct. It should be stated that the earlier investigators worked with *Fucus virsoides* from the Adriatic Sea, whereas both Karrer and Heilbron examined *Fucus vesiculosus,* Karrer obtaining his starting material from Kristineberg (Sweden). The melting point was 159-160°, that of Heilbron 166-168°. Fucoxanthin forms glistening brown prisms, $[\alpha]\frac{18}{\mathrm{Cd}} = +72.5^\circ$ (chloroform), absorption bands 492-457 mμ (chloroform). In the solid state it is stable in the atmosphere, but the orange-yellow solution is very unstable and absorbs oxygen, becoming bleached.

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[341] Karrer, Solmssen: *Helv. Chim. Acta,* **18,** 915 (1935). A pigment from *Euglena heliorubescens* and *Haematococcus pluvialis* termed by Tischer [*Z. physiol. Chem.,* **239,** 257 (1936); **250,** 147 (1938); **252,** 225 (1938)] *euglenarhodone* is identical with astacin; *cf.* Kuhn, Stene, Sörensen: *Ber.,* **72,** 1688 (1939). More recently it has been stated that euglenarhodone gives rise to astacin on saponification: Tischer, *Z. physiol. Chem.,* **267,** 281 (1941). Tischer also describes a new epiphasic haematoxanthin, m.p. 205°, absorption bands 460-560 mμ.

[342] An exhaustive review of the algae pigments is to be found in Klein: "Handbuch der Pflanzenanalyse," **III,** 2, pp. 1382-1410, by Boresch. Prior to fucoxanthin, described above, no pigments of carotenoid character which were analytically confirmed had been described. For this reason no account of them is given in the above description. For the history of the pigments of the *Fucoides,* see Kylin: *Z. physiol. Chem.,* **82,** 221 (1912). For systematic studies of algae pigments see Carter, Heilbron, Lythgoe, *Proc. Roy. Soc.,* **128B,** 82 (1939); Kylin, *K. fysiogr. Sallsk. Lund Forh.,* **7,** 119 (1937); Seybold, Egle, *Jahrb. wiss. Bot.,* **86,** 50 (1938); *see also* Jones, E. R. H., "Ann. Rec. Progr. Pure Chem.," 1940, 302.

[343] Sorby: *Proc. Roy. Soc. London,* **21,** 474 (1873).

[344] Millardet: *Compt. rend.,* **68,** 462 (1869).

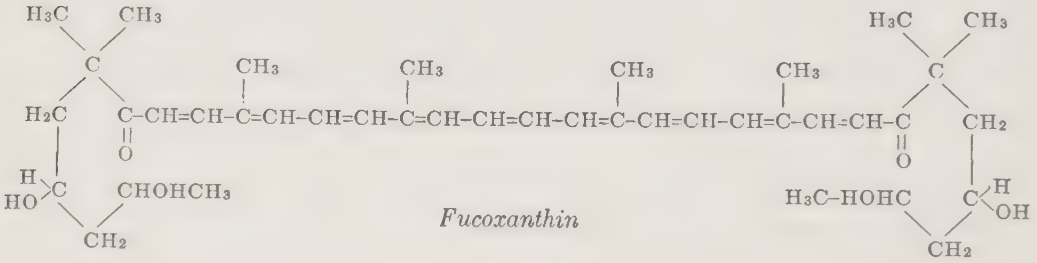
[345] Willstätter, Page: *Ann.,* **404,** 237 (1914); also literature.

[346] Karrer: *Angew. Chemie,* **42,** 918 (1929); Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta,* **14,** 614 (1931).

[347] Heilbron, Phipers: *Biochemical J.,* **29,** 1369 (1935).

Earlier research had shown that the pigment forms an iodide (Willstätter) (violet-black prisms, m.p. 134-135°). Oxidation gives 4.5 mols. of acetic acid and dimethylmalonic acid, but no other dicarboxylic acid can be isolated. The last fact may be understood if both terminal rings are so largely replaced by hydroxyl groups that no dicarboxylic acids more complex than dimethylmalonic acid can remain. On the other hand there appear to be discrepancies in the data available. Thus hydrogenation results in an absorption of hydrogen corresponding to only ten double bonds; and, while Karrer's [348] first Zerewitinoff determinations indicated six hydroxyl groups, his later values [349] record only four or five. Again, on hydrogenation a perhydro-derivative was obtained, the analytical figures of which were intermediate between these for $C_{40}H_{76}O_5$ and for $C_{40}H_{76}O_6$, although the observed number of atoms of active hydrogen is only five. In fucoxanthin itself only two of the hydroxyl groups proved to be esterifiable. It must not be overlooked that fucoxanthin may possibly not be homogeneous and may contain a second component which is poorer in oxygen; it is noteworthy that Willstätter [350] was of the opinion in 1914 that he was dealing with a pigment of the composition $C_{40}H_{56}O_5$ in one of the brown algae.

Heilbron favors the formulation of fucoxanthin as a dihydrocapsorubin:


Fucoxanthin

By the action of alcoholic alkali, products are obtained which Heilbron terms *isofucoxanthins* (aldol condensations products). The color reaction of fucoxanthin is of some importance. With mineral acid it reacts as a weak amine so that an ethereal solution, on shaking with 30-per cent hydrochloric acid, is decolorized, while the acid layer assumes a blue-violet color and contains a stable colored salt having four atoms of chlorine. The same property is encountered in flavoxanthin and violaxanthin.

A simplified preparation of fucoxanthin is described by Karrer.[349] Fresh-water algae [351] particularly the *Chlorophyceae Cladophora Sauteri*,

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[348] Karrer, Wehrli, Helfenstein: *Helv. Chim. Acta*, **13**, 268 (1930); *cf.* Kuhn, Winterstein: *Ber.*, **64**, 330 (1931); Kuhn, Winterstein, Roth: *Ber.*, **64**, 336 (1931).

[349] Karrer, Helfenstein, Wehrli, Pieper, Morf: *Helv. Chim. Acta*, **14**, 614 (1931).

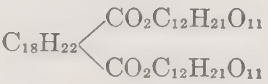
[350] Willstätter, Page: *Ann.*, **404**, 263 (1914).

[351] Heilbron, Parry, Phipers: *Biochem. J.*, **29**, 1376 (1935).

Nitella opaca, Oedogonium and the *Rhodophycea Rhodymenia palmata*, contain lutein, taraxanthin and β-carotene, and in the case of the first mentioned, lactoflavin; β-carotene and zeaxanthin occur in *Halyseris polypoides*.[352]

Crocin. The dried stigma of *Crocus sativus* (saffron), a plant which is indigenous to the Orient but grows also in North Africa, Spain, south of France, Switzerland and Austria, contains a glycoside crocin,[353] lycopene, β-carotene, γ-carotene and zeaxanthin, and finally a bitter principle, *picrocrocin.* Saffron itself is a red-brown or golden-yellow odoriferous powder which finds application as a spice, for coloring foodstuffs and to a smaller extent as a dye for tinting washed yarn.

Crocin [$C_{44}H_{64}O_{26}$, m.p. 186°] may be hydrolyzed into crocetin, a dicarboxylic acid,[354] $C_{20}H_{24}O_4$, and gentiobiose [355] so that it may be represented by



$$C_{18}H_{22}\begin{cases} CO_2C_{12}H_{21}O_{11} \\ CO_2C_{12}H_{21}O_{11} \end{cases}$$

in which both sugar residues appear to be bound as β-glycosides.[356]

Earlier formulas [357] have been superseded, since it is now realized that the original observation of three different hydrolytic products, α-, β-, and γ-crocetin was due to esterification of crocin in contact with methyl or ethyl alcohol,[358] and that the sole primary fission product is *trans*-crocetin or crocetin I (previously known as α-crocetin) [scarlet-red needles, m.p. 283-285°, absorption bands 463-435.5 mμ (chloroform)]. β-Crocetin [rectangular leaflets, m.p. 219°] has the composition $C_{18}H_{22}(CO_2H)CO_2CH_3$ and is thus a monomethylester, and γ-crocetin [hexagonal red-yellow plates, m.p. 222°] is the dimethylester, $C_{18}H_{22}(CO_2CH_3)_2$. This formulation of γ-crocetin has been confirmed by x-ray analysis.

The action of sodium hydroxide on a freshly prepared methyl alcoholic extract of saffron yields not only the above-mentioned crocetin dimethyl ester (m.p. 220°) but also a new crocetin dimethyl ester [359] (m.p. 141°), which is further distinguished from the first by its crystal form, solubility, color and absorption characteristics. It may be converted into the dimethyl ester (m.p. 222°) by the action of light or iodine, or through its dihydrocompound. These are apparently *cis*- and *trans*-isomerides; that derived directly from crocetin I is regarded as the "stable" or *trans* form, and the new dimethyl ester is designated as a "labile" or *cis* form, derived from crocetin II.

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[352] Karrer, Rübel, Strong: *Helv. Chim. Acta*, **19**, 28 (1936).

[353] Older literature: V. Meyer-P. Jacobson: Lehrbuch der organischen Chemie," **II**, 5, 1, p. 175; Karrer, Salomon: *Helv. Chim. Acta*, **10**, 397 (1927).

[354] Kuhn, L'Orsa: *Ber.*, **64**, 1732 (1931).

[355] E. Fischer: *Ber.*, **21**, 988 (1888); Schunck, Marchlewski: *Ann.*, **278**, 357 (1894); Karrer: *Angew. Chemie*, **42**, 919 (1929); Karrer, Miki: *Helv. Chim. Acta*, **12**, 985 (1929); Kuhn, Winterstein: *Naturwiss.*, **21**, 527 (1933); *Ber.*, **67**, 344 (1934).

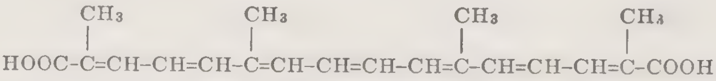
[356] Kuhn, Wang: *Ber.*, **72**, 871 (1939), where the synthesis of a tetradeca-acetyl crocin is described.

[357] Karrer, Salomon: *Helv. Chim. Acta*, **10**, 397 (1927); Karrer, Helfenstein, Widmer: *Ibid.*, **11**, 1201 (1928).

[358] Karrer, Helfenstein: *Ibid.*, **13**, 392 (1930).

[359] Kuhn, Winterstein: *Ber.*, **66**, 209 (1933).

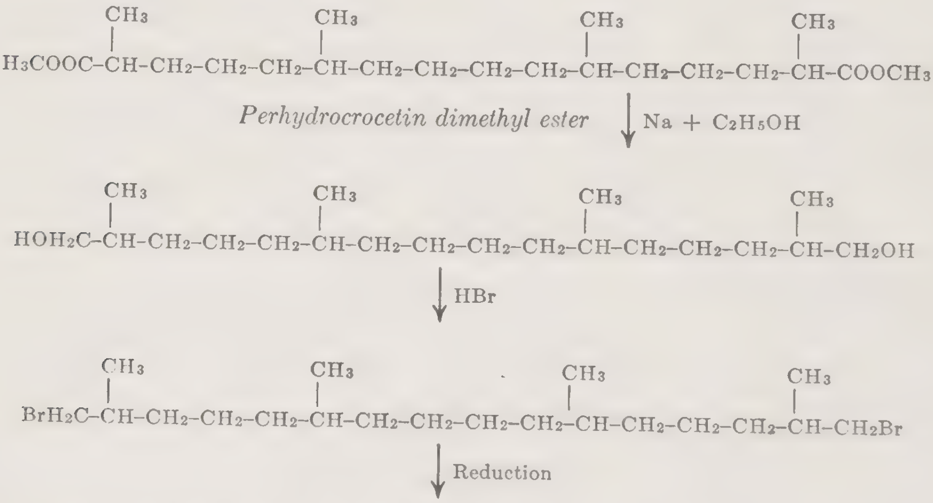
Crocetin I may be partially reduced to a dihydrocompound[360] (m.p. 192-193°) by titanium chloride, and on exhaustive hydrogenation takes up 7 mols. of hydrogen. Oxidative degradation with chromic acid[354] yields 3.53 mols. of acetic acid, but as degradation with permanganate[361] yields only 3 mols. of acetic acid, it was concluded that the fourth methyl group is directly attached to a carboxyl group; permanganate degradation would then lead to the intermediate formation of pyruvic acid, which under the experimental conditions obtaining would yield practically no acetic acid.[362] After several unsatisfactory structures had been proposed, the following constitution, which satisfies all the experimental results and theoretical considerations, was suggested:[363]



$$\mathrm{HOOC{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}COOH}$$

Crocetin I

Crocetin I may be converted into the saturated hydrocarbon[364] crocetane, 2,6,11,15-tetramethylhexadecane, by hydrogenation and subsequent degradation:



$$\mathrm{H_3COOC{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}COOCH_3}$$

Perhydrocrocetin dimethyl ester

$$\Big\downarrow \mathrm{Na + C_2H_5OH}$$

$$\mathrm{HOH_2C{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2OH}$$

$$\Big\downarrow \mathrm{HBr}$$

$$\mathrm{BrH_2C{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2Br}$$

$$\Big\downarrow \text{Reduction}$$

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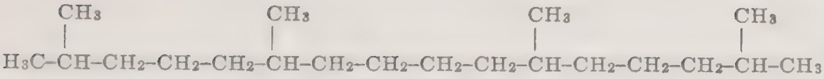
[360] The dihydrocompound may be oxidized to crocetin by oxygen in presence of catalytic bases:

[361] Kuhn, Winterstein, Karlowitz: *Helv. Chim. Acta*, **12**, 64 (1929).

[362] Karrer, Benz, Morf, Raudnitz, Stoll, Takahashi: *Helv. Chim. Acta*, **15**, 1399 (1932). Kuhn, Drumm: *Ber.*, **65**, 1458 (1932).

[363] Karrer, Benz, Morf, Raudnitz, Stoll, Takahashi, *Ibid.*, **15**, 1218, 1399 (1932).

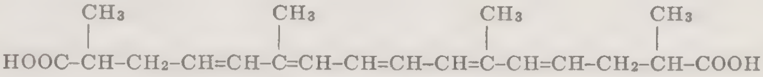
[364] Karrer, Golde: *Ibid.*, **13**, 707 (1930); according to a later paper [Karrer, *et al.*, ref. 362, p. 1406] the constitutions given in 1930 require modification. Kuhn, Grundmann [*Ber.*, **70**, 1318 (1937)] would employ here also the nomenclature of the other carotenoid pigments so that *apo*, in conjunction with the trivial name of the pigment under discussion, would signify the lack of a methyl group and *des* the lack of all substituting methyl groups. See also the synthesis of des-crocetin.



$$H_3C-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_3$$

Crocetane

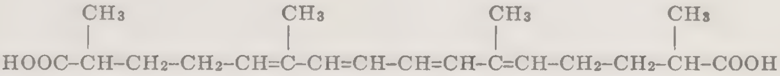
The constitution assigned to crocetin is further confirmed by oxidation experiments on tetrahydrocrocetin:



$$HOOC-\overset{CH_3}{CH}-CH_2-CH{=}CH-\overset{CH_3}{C}{=}CH-CH{=}CH-CH{=}\overset{CH_3}{C}-CH{=}CH-CH_2-\overset{CH_3}{CH}-COOH$$

Tetrahydrocrocetin

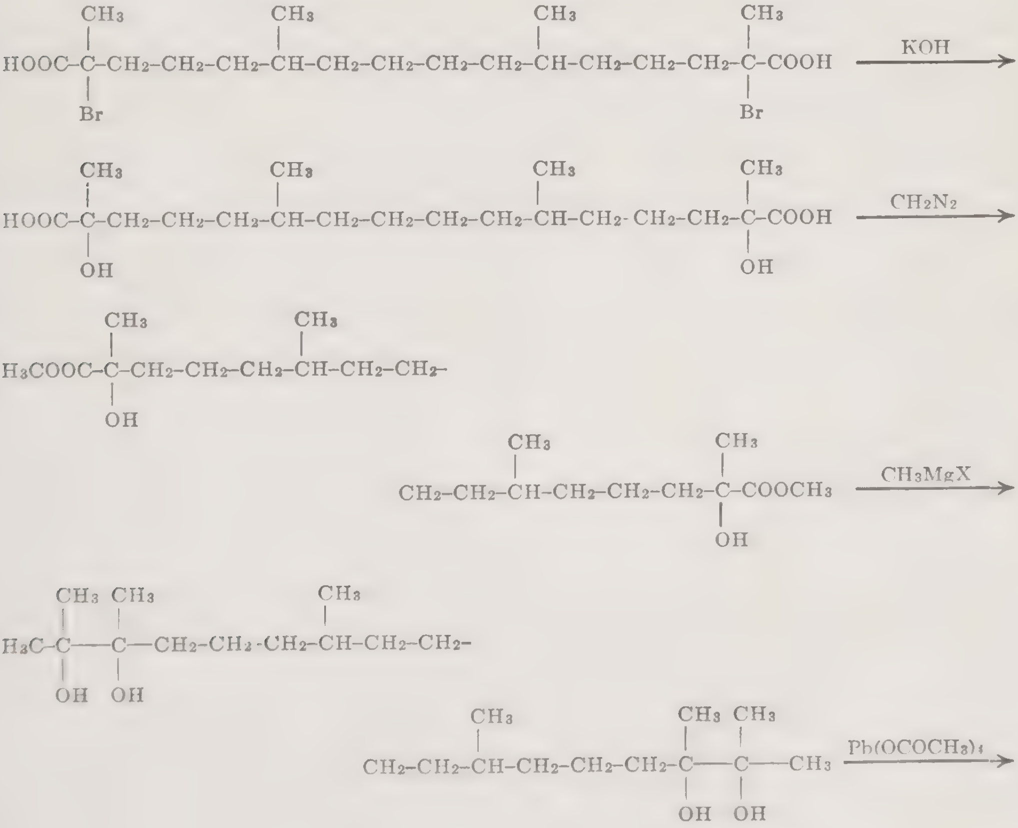
and hexahydrocrocetin



$$HOOC-\overset{CH_3}{CH}-CH_2-CH_2-CH{=}\overset{CH_3}{C}-CH{=}CH-CH{=}CH-\overset{CH_3}{C}{=}CH-CH_2-CH_2-\overset{CH_3}{CH}-COOH$$

Hexahydrocrocetin

with potassium permanganate. Tetrahydrocrocetin should yield methylsuccinic acid and hexahydrocrocetin α-methylglutaric acid, and this was in fact observed. Again, perhydrocrocetin was degraded by the following series of reactions to a diketone[363]:

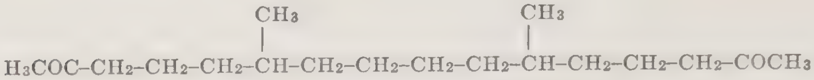


$$HOOC-\underset{Br}{\overset{CH_3}{C}}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\underset{Br}{\overset{CH_3}{C}}-COOH \xrightarrow{KOH}$$

$$HOOC-\underset{OH}{\overset{CH_3}{C}}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\underset{OH}{\overset{CH_3}{C}}-COOH \xrightarrow{CH_2N_2}$$

$$H_3COOC-\underset{OH}{\overset{CH_3}{C}}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-$$
$$CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\underset{OH}{\overset{CH_3}{C}}-COOCH_3 \xrightarrow{CH_3MgX}$$

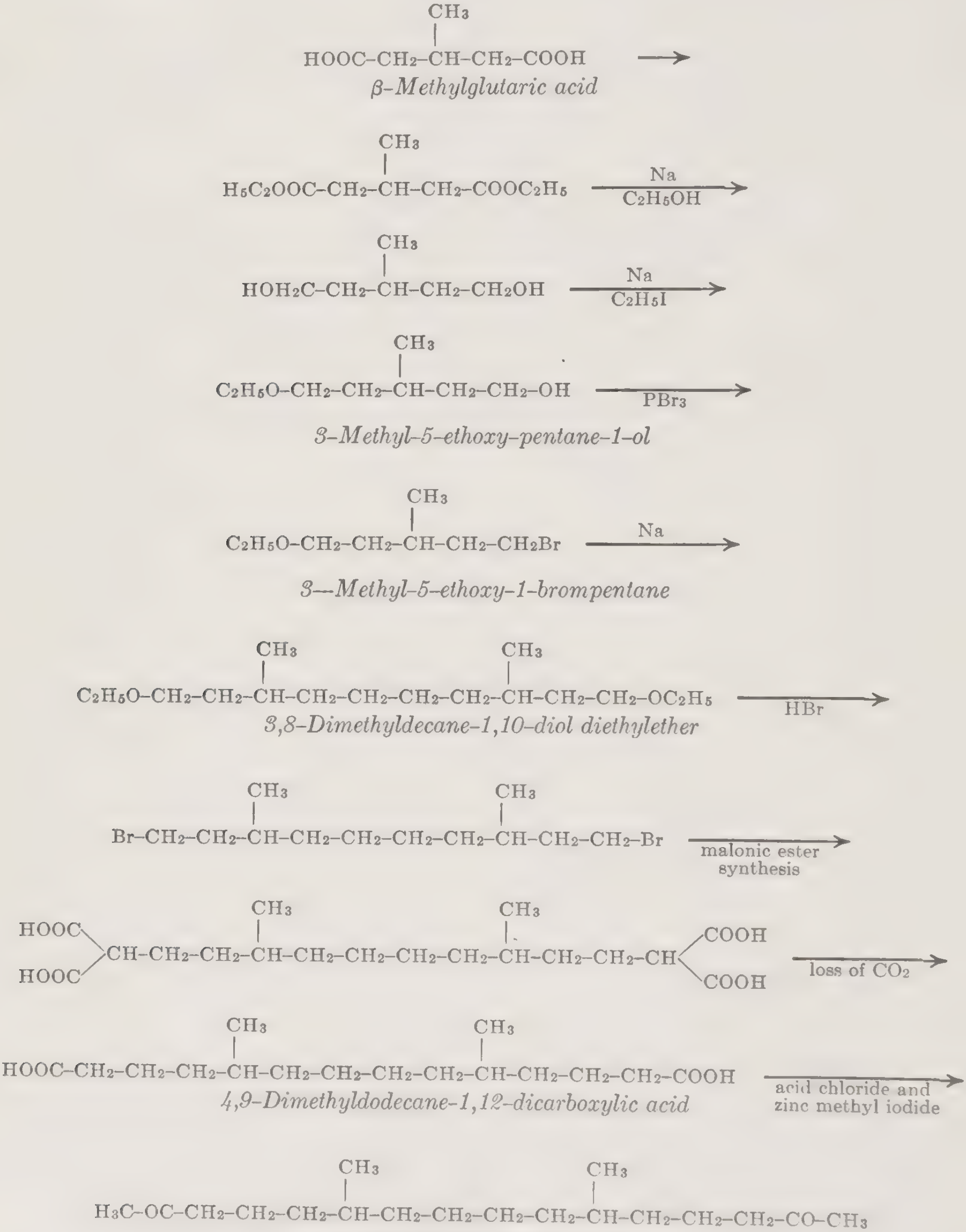
$$H_3C-\underset{OH}{\overset{CH_3}{C}}-\underset{OH}{\overset{CH_3}{C}}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-$$
$$CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\underset{OH}{\overset{CH_3}{C}}-\underset{OH}{\overset{CH_3}{C}}-CH_3 \xrightarrow{Pb(OCOCH_3)_4}$$



$$H_3COC{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}COCH_3$$

6,11-Dimethylhexadecane-2,15-dione

and the synthesis[365] of the last compound was accomplished in the following manner:



$$HOOC{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}COOH \longrightarrow$$

β-Methylglutaric acid

$$H_5C_2OOC{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}COOC_2H_5 \xrightarrow[C_2H_5OH]{Na}$$

$$HOH_2C{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2OH \xrightarrow[C_2H_5I]{Na}$$

$$C_2H_5O{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}OH \xrightarrow[PBr_3]{}$$

3-Methyl-5-ethoxy-pentane-1-ol

$$C_2H_5O{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2Br \xrightarrow{Na}$$

3—Methyl-5-ethoxy-1-brompentane

$$C_2H_5O{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}OC_2H_5 \xrightarrow[HBr]{}$$

3,8-Dimethyldecane-1,10-diol diethylether

$$Br{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}Br \xrightarrow[\text{malonic ester synthesis}]{}$$

$$\begin{matrix}HOOC\\HOOC\end{matrix}\!\!>\!CH{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH\!<\!\begin{matrix}COOH\\COOH\end{matrix} \xrightarrow[\text{loss of } CO_2]{}$$

$$HOOC{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}COOH \xrightarrow[\text{acid chloride and zinc methyl iodide}]{}$$

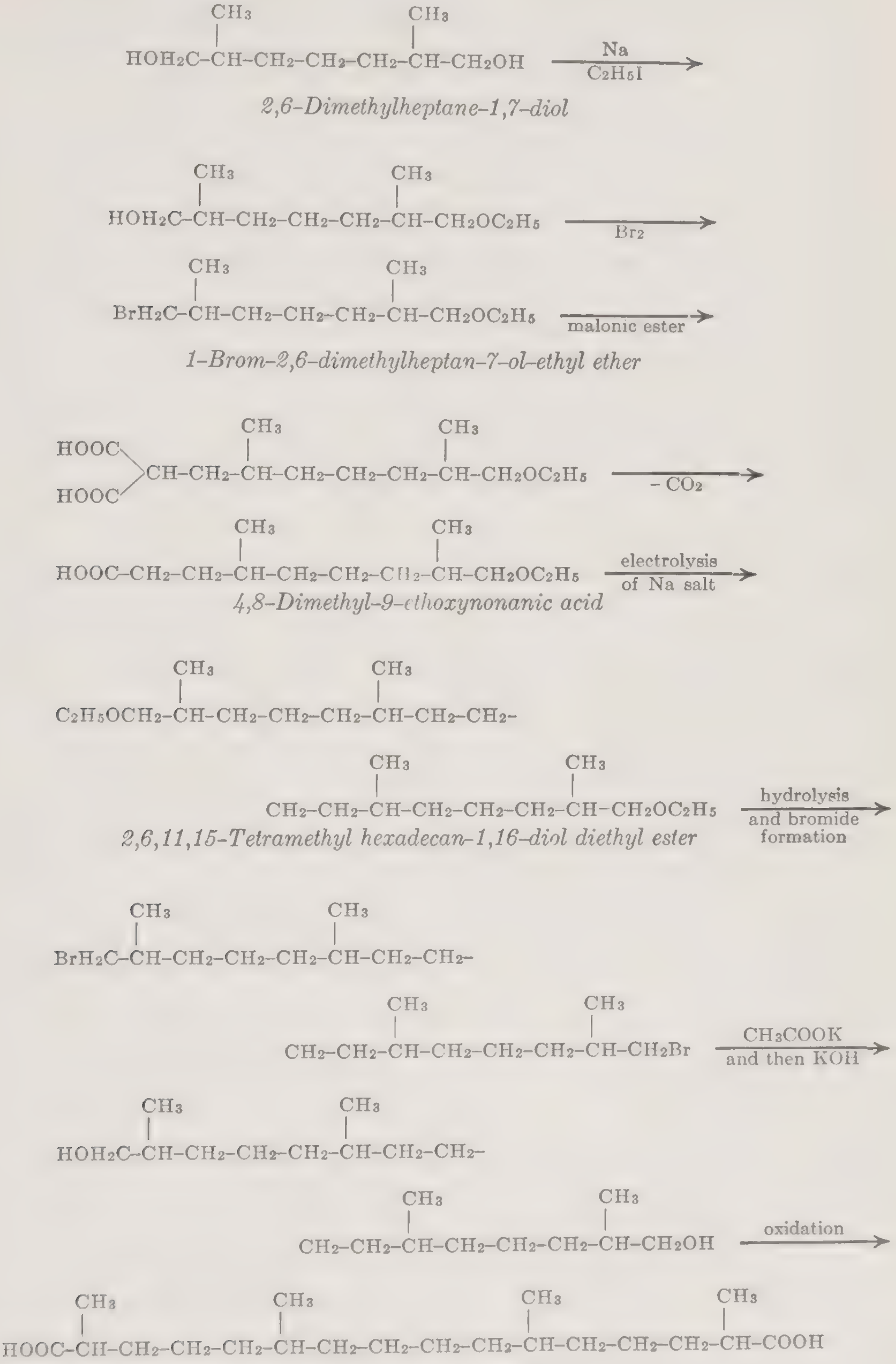
4,9-Dimethyldodecane-1,12-dicarboxylic acid

$$H_3C{-}OC{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}CO{-}CH_3$$

6,11-Dimethylhexadecane-2,15-dione

[365] Karrer, Lee: *Helv. Chim. Acta,* 17, 543 (1934).

Finally perhydrocrocetin has been synthesized.[366]



$$HOH_2C-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OH \xrightarrow[C_2H_5I]{Na}$$

2,6-Dimethylheptane-1,7-diol

$$HOH_2C-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OC_2H_5 \xrightarrow[Br_2]{}$$

$$BrH_2C-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OC_2H_5 \xrightarrow[\text{malonic ester}]{}$$

1-Brom-2,6-dimethylheptan-7-ol-ethyl ether

$$\begin{matrix}HOOC\\HOOC\end{matrix}\rangle CH-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OC_2H_5 \xrightarrow[-CO_2]{}$$

$$HOOC-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OC_2H_5 \xrightarrow[\text{of Na salt}]{\text{electrolysis}}$$

4,8-Dimethyl-9-ethoxynonanic acid

$$C_2H_5OCH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-$$

$$CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OC_2H_5 \xrightarrow[\text{and bromide formation}]{\text{hydrolysis}}$$

2,6,11,15-Tetramethyl hexadecan-1,16-diol diethyl ester

$$BrH_2C-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-$$

$$CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2Br \xrightarrow[\text{and then KOH}]{CH_3COOK}$$

$$HOH_2C-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-$$

$$CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2OH \xrightarrow{\text{oxidation}}$$

$$HOOC-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}H-COOH$$

Perhydrocrocetin

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[366] Karrer, Benz, Stoll: *Helv. Chim. Acta*, **16**, 297 (1933).

For the relationship between crocetin and bixin, see later under bixin.

Crocetin dimethyl ester [367] yields 10 per cent of toluene and *m*-xylene, 12 per cent of the dimethylester of 1,4,8-trimethyloctatetraene-1,8-dicarboxylic acid, $C_{15}H_{20}O_4$, and 1 per cent of tricyclocrocetin on thermal degradation.

The isomerization of crocetin has recently acquired an extraordinary interest from the recognition of the part which it plays in bringing about sexual reproduction in some green algae.

The function of echinochrome and some other similar pigments of the naphthalene series has already been mentioned. These are chemotactic agents, chemical messengers secreted by the female organism and serving to attract the spermatozoa. In strains of *Chlamydomonas eugametos* studied by Kuhn [*Angew. Chem.,* **53, 1** (1940); lecture giving also the literature] minute amounts of another material produce a superficially similar effect. The phenomena are demonstrated by illuminating a suspension of the gametes and then filtering. The flagellate gametes (*Chlamydomonas* is an isogamous organism) are motile in light but quiescent in the dark. Gametes in a "dark" suspension become motile, however, when treated with a suspension from an illuminated (motile) gamete suspension. The explanation is that the motile gametes secrete crocin into the surrounding solution, and quantitative experiment shows that a gamete is rendered motile by approximately one molecule of crocin. Motility, however, does not alone insure fertilization, and this only follows on further irradiation—even of the cell-free filtrate which can then be added to the gametes. With any one strain of *Chlamydomonas eugametos* there is reached a stage when the irradiated solution will render female gametes able to copulate; on more prolonged irradiation this power is lost, but the solution can now render male cells able to copulate; on still further irradiation the solution becomes permanently inactive. All these phenomena can be reproduced by mixtures of *cis*- and *trans*-crocetin dimethyl ester (the effect is specific and the effect is not shown by the free acid or even the ethyl ester). With *Chlamydomonas eugametos f. simplex,* the female gametes are activated by a mixture of 3 parts of *cis* and 1 part *trans,* the male gametes by a mixture of 1 part of *cis* and 3 parts of *trans* ester; the pigments are to be detected by this means at a dilution of 1:33,000,000,000. The ratio *cis*:*trans* = 75:25 applies only to the *simplex* strain. Others have another ratio but the "female" and "male" ratios are complementary:

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[367] Kuhn, Winterstein: *Ber.,* **65,** 1873 (1932); **66,** 1733 (1933).

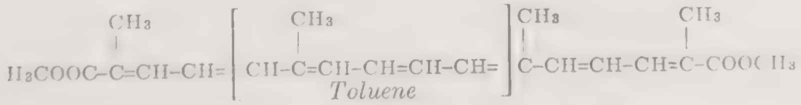
Chlamydomonas Braunii	♀^{4} 95% *cis*, 5% *trans*
Chlamydomonas Dresdensii, C. eug. f. typica	♀^{3} 85% *cis*, 15% *trans*
C. eugametos f. simplex	♀^{2} 75% *cis*, 25% *trans*
C. eugametos f. synoica, f.	♀^{1} 65% *cis*, 35% *trans*
subheteroica	♂^{1} 35% *cis*, 65% *trans*
C. eugametos f. simplex	♂^{2} 25% *cis*, 75% *trans*
C. eugametos f. typica	♂^{3} 15% *cis*, 85% *trans*
C. Braunii	♂^{4} 5% *cis*, 95% *trans*

An extraordinary result emerges from this table. In certain cases male gametes may copulate with male gametes of another strain and female with female. These cases (X) are shown in the following table where normal copulation is indicated by x. It seems then that copulation between two partners is decided by the composition of the *cis-trans* crocetin mixture which activates them; for copulation to occur the difference between the amount of *cis* and *trans* isomerides must amount to at least 20 per cent. Here is the phenomenon of "relative sexuality," and for the first time a chemical basis is provided for it.

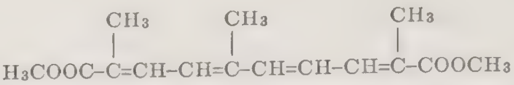
	♀^{4}	♀^{3}	♀^{2}	♀^{1}	♂^{1}	♂^{2}	♂^{3}	♂^{4}
♀^{4}	..	..	X	X	x	x	x	x
♀^{3}	..	..	..	X	x	x	x	x
♀^{2}	X	..	..	..	x	x	x	x
♀^{1}	X	X	..	..	x	x	x	x
♂^{1}	x	x	x	x	..	..	X	X
♂^{2}	x	x	x	x	..	..	..	X
♂^{3}	x	x	x	x	X	..	..	..
♂^{4}	x	x	x	x	X	X	..	..

Something also is known of the sex-determining activity (for *Chlamydomonas*) of the glycoside picrocrocin which it will be remembered is associated with crocin in saffron; this, not belonging to color chemistry, is not dealt with at length here. It need only be remarked that the photoisomerization of other carotenoids may also be associated with biological activity. The occurrence of crocin and picrocrocin in the reproductive apparatus of flowers suggests that they are of fundamental importance here also, and there is no doubt that the incomplete account which it is possible to give at the present time is only the beginning of what promises to be the most fascinating story in chemistry.

Trimethyloctatetraene-1,8-dicarboxylic acid (yellow prisms, m.p. 135°) is a lower homolog of crocetin (and also of bixin), and may be regarded as owing its formation to the loss of the elements of 1 mol. of toluene from the central part of the molecule of crocetin dimethyl ester and subsequent linking of the terminal residues:



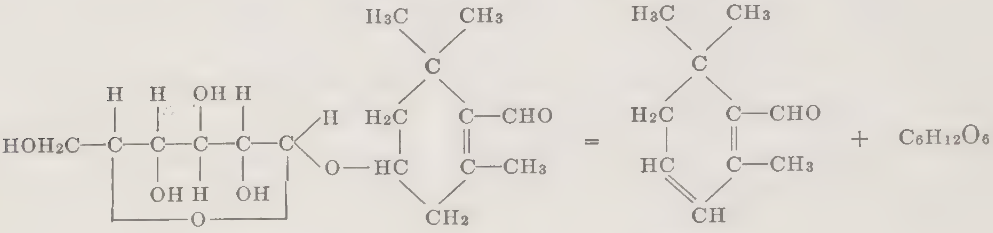
$$H_3COOC-\overset{CH_3}{\overset{|}{C}}=CH-CH=\left[\underset{\textit{Toluene}}{CH-\overset{CH_3}{\overset{|}{C}}=CH-CH=CH-CH=}\right]\overset{CH_3}{\overset{|}{C}}-CH=CH-CH=\overset{CH_3}{\overset{|}{C}}-COOCH_3$$



$$H_3COOC-\overset{CH_3}{\overset{|}{C}}=CH-CH=\overset{CH_3}{\overset{|}{C}}-CH=CH-CH=\overset{CH_3}{\overset{|}{C}}-COOCH_3$$

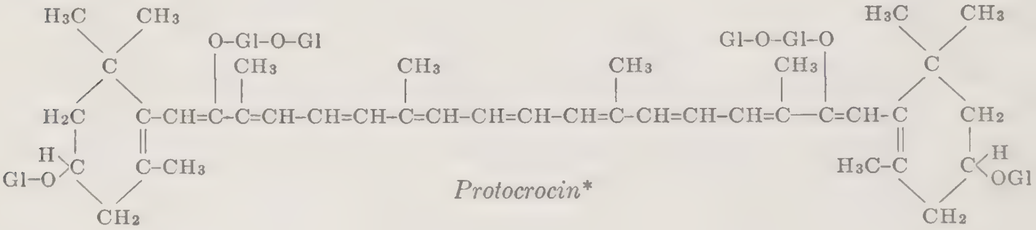
1,4,8-Trimethyloctatetraene-1,8-dicarboxylic methyl ester

Tricyclocrocetin [$C_{20}H_{24}O_4$, colorless needles, mp. 263-264°] is isomeric with crocetin but takes up only 4 instead of 7 mols. of hydrogen and hence contains 3 carbocyclic rings. The bitter principle of saffron,[368] picrocrocin, $C_{16}H_{26}O_7$, is a glucoside derived from glucose and safranal [369] and, according to Kuhn and Winterstein,[368] has the constitution:

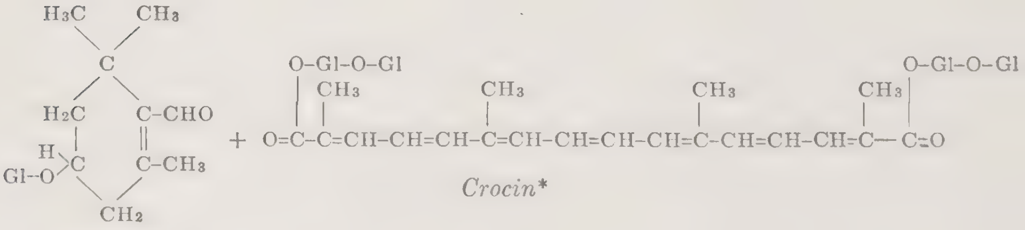


Picrocrocin *Safranal*

This constitution is noteworthy as it adds plausibility to the assumption that the group of carotenoid acids to which crocetin belongs arise in the plant by enzymic degradation [370] of the carotenoid pigments containing 40 carbon atoms, picrocrocin originating as the terminal group of a "protocrocin."



*Protocrocin**



*Picrocrocin** *Crocin**

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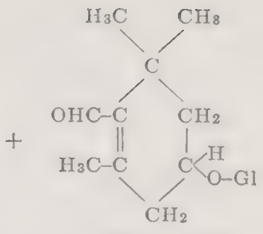
[368] Kuhn, Winterstein: *Ber.*, **67**, 344 (1934).

[369] Synthesis: Kuhn, Wendt: *Ber.*, **69**, 1549 (1936).

[370] Kuhn, Winterstein: *Ber.*, **65**, 646 (1932); Kuhn, Grundmann: *Ber.*, **65**, 1880 (1932); Kuhn, Deutsch: *Ber.*, **66**, 883 (1933).

* Gl = Glucose; —Gl—O—Gl = Gentiobiose.



*Picrocrocin**

On this assumption 1 mol. of crocin should yield 2 mols. of picrocrocin, 1.4 mol. being in fact found; the difference may be explained by the sensitiveness of the terpene aldehyde glucoside, the amount of which in saffron diminishes on standing.

The postulated mechanism of the formation of carotenoid pigments in the plant by union of isoprene residues with simultaneous or subsequent dehydrogenation finds some support in the observation that the dimethyl ester of dihydrocrocetin may be dehydrogenated to crocetin dimethyl ester[371] on shaking with air in the presence of catalytic bases. The dihydro ester gives blue solutions with alkali in certain solvents, and these, on being shaken with air, become orange-red, with formation of the dimethyl ester of crocetin.[372]

Crocetin[373] has been found in the Chinese pigment "wongsky" obtained from the fruit of *Gardenia grandiflora.*[374] It is identical with *nictanthin,*[375] the coloring matter of the corolla of *Nyctanthes arbor tristis,*[376] a shrub of the *Oleaceae* family growing in the Himalayas, and occurs also in the blooms of *Cedrala toona,*[377] the Indian mahogany tree (Himalayas, central and south India, Java, Australia), known in this form as pigment under the name "gunari," and also in torch-weed.[378] It is best prepared from saffron by the method due to Karrer,[379] due attention being paid to the readiness with which esterification occurs.

Bixin[380] is the pigment of the red waxy material which surrounds the seeds of the tropical tree, *Bixa orellana,* and which is known under the names of orlean, rocou, anotto or annatto, orenetto, attalo and terra orellana. It has the empirical composition[381] $C_{25}H_{30}O_4$, first established by Heiduschka and Panzer,[382] and forms red-brown rhombic crystals [m.p. 198°, absorption bands 503-469.5-439 mμ (chloroform)]. A determination of the molecular weight by x-ray methods has been carried out on methylbixin [383] $C_{26}H_{32}O_4$.

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* Gl = Glucose; —Gl—O—Gl = Gentiobiose.

[371] Kuhn, Drumm: *Ber.*, **65**, 1458 (1932).

[372] Kuhn, Drumm, Hoffer, Möller: *Ber.*, **65**, 1785 (1932).

[373] Rochleder: *J. prakt. Chem.*, **72**, 394 (1857); Rochleder, Mayer: Ibid., **74**, 1 (1858); Munesada: *J. Soc. Pharm. Japan,* No. **486,** 1, (1922).

[374] Kuhn, Winterstein, Wiegand: *Helv. Chim. Acta,* **11,** 716 (1928).

[375] Hill, Sirkar: *J. Chem. Soc.*, **91,** 1501 (1907).

[376] Kuhn, Winterstein, Wiegand: *Helv. Chim. Acta,* **11,** 716 (1928); Kuhn, Winterstein: *Ibid.*, **12,** 496 (1929).

[377] Perkin: *J. Chem. Soc.*, **101,** 1538 (1912).

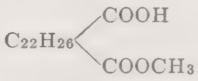
[378] Schmid, Kotter: *Monatsh.*, **59,** 341 (1932).

[379] Karrer, Helfenstein: *Helv. Chim. Acta,* **13,** 392 (1930).

[380] Older literature: V. Meyer and P. Jacobson: "Lehrbuch der organischen Chemie," **II,** 5, 1, p. 177, 178; Zwick: *Arch. Pharm.*, 238, 58 (1900).

[381] Errors and confusion arising in the deduction of the formula of bixin are described in reference 380; older formulas are reviewed by Karrer, Helfenstein, Widmer, van Itallie: *Helv. Chim. Acta,* **12,** 741 (1929).

Earlier researches had established that bixin contains a hydroxyl and a methoxyl group,[384] as on treating with dilute potassium hydroxide, a monopotassium salt is first obtained and a dipotassium salt is more slowly formed. The latter, with acid, yields not bixin but a new compound, norbixin (pale red crystals, m.p. 250-255°) which no longer contains a methoxyl group. On the other hand bixin may be methylated to give methylbixin (m.p. 163-164°) so that it is probable that both hydroxyls belong to carboxyl groups and that the bixin formula may be partially elucidated as:



$$C_{22}H_{26}\left\langle\begin{matrix}COOH\\COOCH_3\end{matrix}\right.$$

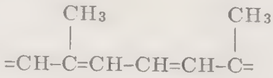
However, the two carboxyl groups are not equivalent, as two different methyl ethyl esters have been prepared.

Hydrogenation [385] results in an uptake of 9 mols. of hydrogen, and methylbixin on ozonolysis yields methylglyoxal and methyl β-acetylacrylic ester,

$$CH_3CO{-}CH{=}CH{-}COOCH_3$$

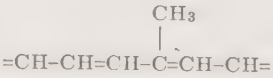
the last apparently originating as a terminal group.

Finally thermal decomposition [387] gives rise to *m*-xylene, toluene, *m*-toluic acid and its methyl ester. In devising a formula for bixin it must be assumed that *m*-xylene corresponds to the grouping:



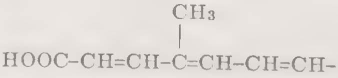
$$=CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}$$

toluene to the group:



$$=CH{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}$$

m-toluic acid to the group:



$$HOOC{-}CH{=}CH{-}\overset{\overset{CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}$$

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[382] Heiduschka, Panzer: *Ber.*, 50, 546, 1525 (1917).

[383] Hengstenberg, Kuhn: *Z. Krystallogr.*, 76, 174 (1931).

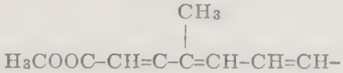
[384] van Hasselt: *Chem. Weekblad*, 6, 480 (1909).

[385] Liebermann, Mühle: *Ber.*, 48, 1653 (1915); Herzig, Faltis: *Ann.*, 431, 40 (1923).

[386] Rinkes: *Chem. Weekblad*, 12, 996 (1915); Rinkes, van Hasselt: *Ibid.*, 13, 436 (1916); Rinkes: *Rec. trav. chim.*, 47, 934 (1928).

[387] van Hasselt: *Chem. Weekblad*, 6, 480 (1909); Kuhn, Winterstein: *Ber.*, 65, 1873 (1932); *cf.* Kuhn, Deutsch: *Ber.*, 65, 43 (1932).

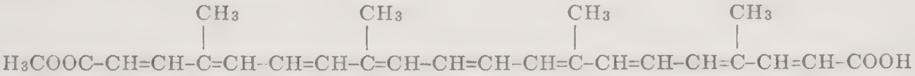
and *m*-toluic methyl ester to the group:



$$H_3COOC{-}CH{=}C{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}$$

Of the 25 carbon atoms of bixin, 19 (9 in methyl *m*-toluate, 8 in *m*-toluic acid and at least 2 more in *m*-xylene) are thus accounted for. The similarity between bixin and crocetin [388] was noted quite early.

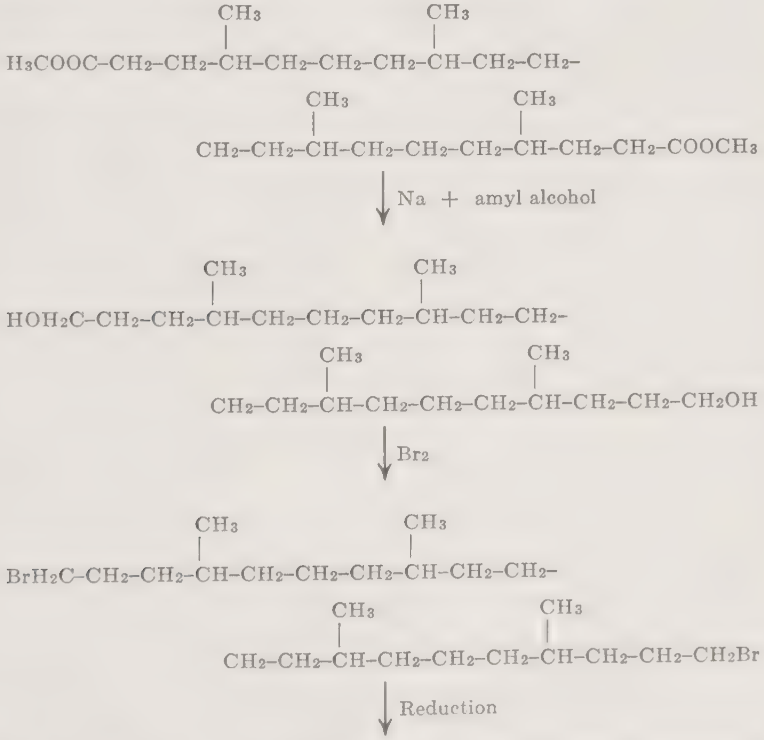
Determination of the nature of the side-chains [389] of bixin with the aid of an oxidative method using potassium permanganate, which yields 4 mols. of acetic acid, leads to the following formula,[390] which provides an explanation of all the foregoing facts and supersedes the older symmetrical formulas:



$$H_3COOC{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}COOH$$

Bixin

The dimethyl ester of perhydrobixin [391] has been reduced with sodium and amyl alcohol to a diprimary glycol which was converted into the parent hydrocarbon, bixane ($C_{24}H_{50}$, b.p. 218°/15 mm.):



$$\begin{array}{l}
H_3COOC{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}\\
\qquad CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}COOCH_3\\
\qquad\qquad \downarrow \text{Na + amyl alcohol}\\
HOH_2C{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}\\
\qquad CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2OH\\
\qquad\qquad \downarrow Br_2\\
BrH_2C{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}\\
\qquad CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}H}{-}CH_2{-}CH_2{-}CH_2Br\\
\qquad\qquad \downarrow \text{Reduction}
\end{array}$$

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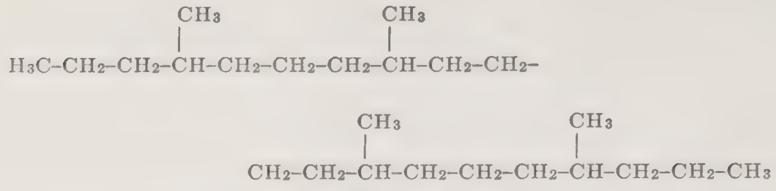
[388] Karrer, Salomon: *Helv. Chim. Acta*, 11, 513 (1928); History of the determination of the constitution: Kuhn, Winterstein, Wiegand: *Ibid.*, 11, 716 (1928).

[389] Kuhn, Winterstein, Karlowitz: *Ibid.*, 12, 64 (1929).

[390] Kuhn, Winterstein: *Ber.*, 65, 646, 1873 (1932).

[391] Kuhn, Ehmann: *Helv. Chim. Acta*, 12, 904 (1929); the constitutional formula of Faltis and Vieböck [*Ber.*, 62, 701 (1929)] is here refuted; see also Kuhn, Winterstein: *Helv. Chim. Acta*, 12, 899 (1929), and Karrer, Helfenstein, Widmer, van Itallie: *Ibid.*, 12, 741 (1929).



$$H_3C-CH_2-CH_2-\overset{\overset{\displaystyle CH_3}{|}}{C}H-CH_2-CH_2-CH_2-\overset{\overset{\displaystyle CH_3}{|}}{C}H-CH_2-CH_2-$$

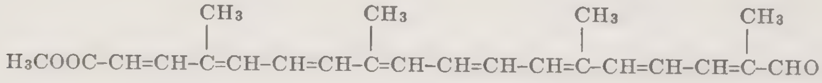
$$CH_2-CH_2-\overset{\overset{\displaystyle CH_3}{|}}{C}H-CH_2-CH_2-CH_2-\overset{\overset{\displaystyle CH_3}{|}}{C}H-CH_2-CH_2-CH_3$$

Bixane

As already mentioned, the two carboxyl groups of bixin are not equivalent. The view was formerly held that this difference is due to structural isomerism [392] in the halves of the bixin molecule; but newer knowledge has shown that only an unsymmetrical *cis*-linkage [393] is concerned in the distinction between the carboxylic groups, and that the structure is otherwise symmetrical. This view is confirmed by the existence of a β-bixin (bixin I)[394] which is regarded [395] as a *trans* isomeride, as both ordinary bixin (bixin II) and bixin I may be converted into the same dihydrocompound.[396]

Bixin II (*cis*-form) yields 3 aldehydes on controlled degradation with potassium permanganate [397]:

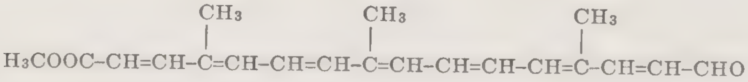
I.



$$H_3COOC-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-CH=\overset{\overset{\displaystyle CH_3}{|}}{C}-CH=CH-CH=\overset{\overset{\displaystyle CH_3}{|}}{C}-CHO$$

Apo-1-norbixinal methyl ester, $C_{23}H_{28}O_3$, orange leaves, m.p. 156°; oxime $C_{23}H_{29}O_3N$, leaflets, m.p. 186°.

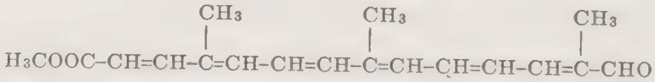
II.



$$H_3COOC-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-CH=\overset{\overset{\displaystyle CH_3}{|}}{C}-CH=CH-CHO$$

Apo-2-norbixinal methyl ester [absorption bands 445-427 m$\bar{\mu}$ (CS_2)] is obtained in only small amount.

III.



$$H_3COOC-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-\overset{\overset{\displaystyle CH_3}{|}}{C}=CH-CH=CH-CH=\overset{\overset{\displaystyle CH_3}{|}}{C}-CHO$$

Apo-3-norbixinal methyl ester, $C_{18}H_{22}O_3$, orange clusters, m.p. 188°; semicarbazone, $C_{19}H_{25}O_3N_3$, m.p. 215°.

Bixin I (*trans* bixin) yields an apo-1-norbixinal methyl ester [m.p. 167° (oxime, m.p. 196°)] which may also be obtained from the isomeride

[392] Herzig, Faltis: *Ann.*, **431,** 40 (1923); Kuhn, Winterstein: *Helv. Chim. Acta*, **11,** 427 (1928).

[393] Kuhn, Winterstein: *Ber.*, **65,** 646, 1873 (1932).

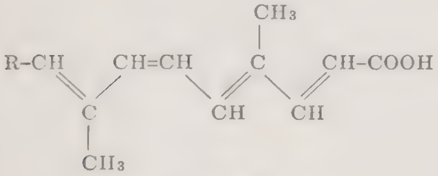
[394] Herzig, Faltis: *Ann.*, **431,** 40 (1923).

[395] Karrer, Helfenstein, Widmer, van Itallie: *Helv. Chim. Acta*, **12,** 741 (1929); termed bixin I and II: Kuhn, Winterstein: *Ber.*, **66,** 209 (1933); Karrer, Takahashi: *Helv. Chim. Acta*, **16,** 287 (1933).

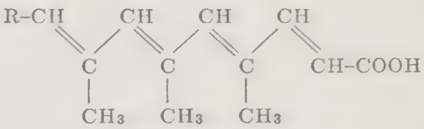
[396] Kuhn, Winterstein: *Ber.*, **65,** 646 (1932).

[397] Karrer, Solmssen: *Helv. Chim. Acta*, **20,** 1396 (1937).

above by treating with iodine in ethyl acetate solution. The corresponding apo-2-norbixinal methyl ester from bixin I is, from spectroscopic data, not identical with that above, but both bixin I and bixin II furnish the same apo-3-norbixinal methyl ester. The isomerism of the two bixins is thus ascribed to different configurations about the third ethylenic linkage from the unesterified carboxyl group:

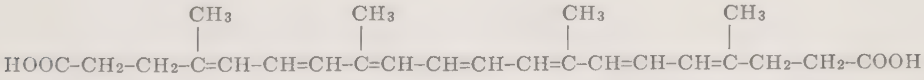


Bixin II
cis (labile) form



Bixin I
trans (stable) form

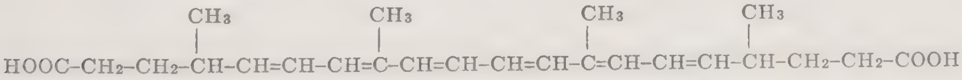
Consideration of this structure reveals that bixin and the central part of the lycopene molecule are constituted in the same way. That the structure of bixin is a symmetrical one, moreover (neglecting the spatial arrangement mentioned above), may be deduced from the following facts.[398] Norbixin was reduced by sodium amalgam to tetrahydronorbixin:



$$\mathrm{HOOC{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH_2{-}CH_2{-}COOH}$$

Tetrahydronorbixin

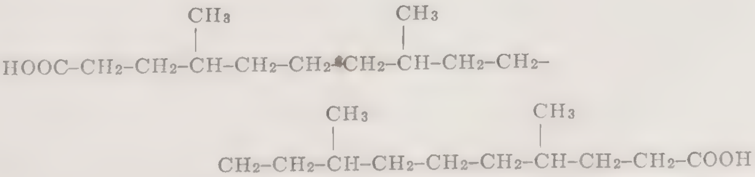
and to hexahydronorbixin:



$$\mathrm{HOOC{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH{=}CH{-}CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}COOH}$$

Hexahydronorbixin

of which the former yielded succinic acid and the latter α-methyl glutaric acid on oxidation with potassium permanganate. Again, perhydronorbixin was degraded to a dialdehyde, 3,7,12,16-tetramethyloctadecane-1,18-dialdehyde (b.p. 185° at 0.6 mm.):



$$\mathrm{HOOC{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}}$$
$$\mathrm{CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}COOH}$$

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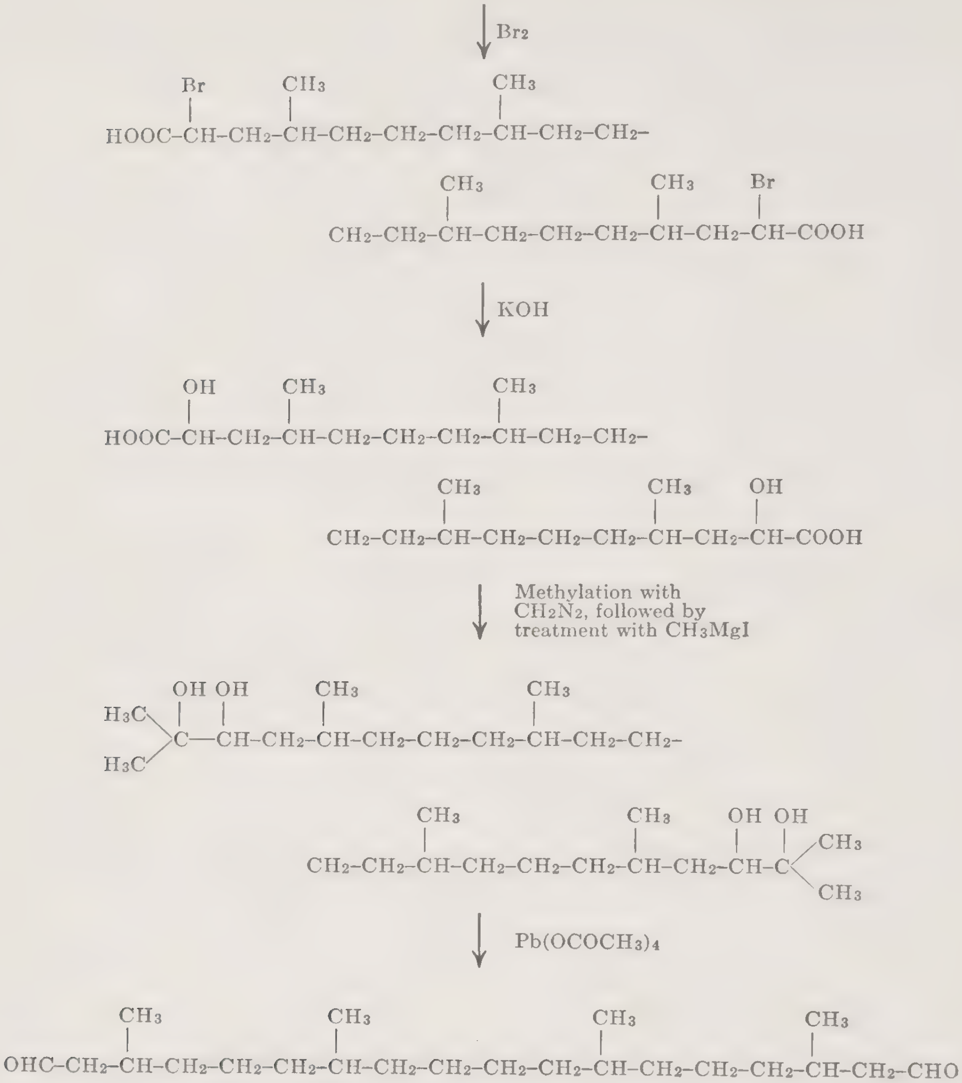
[398] Karrer, Benz, Morf, Raudnitz, Stoll, Takahashi: *Helv. Chim. Acta*, **15**, 1218, 1399 (1932).



$$\Big\downarrow \text{Br}_2$$

$$HOOC-\overset{Br}{CH}-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-$$

$$CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-\overset{Br}{CH}-COOH$$

$$\Big\downarrow \text{KOH}$$

$$HOOC-\overset{OH}{CH}-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-$$

$$CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-\overset{OH}{CH}-COOH$$

$$\Big\downarrow \begin{array}{l}\text{Methylation with}\\ CH_2N_2\text{, followed by}\\ \text{treatment with } CH_3MgI\end{array}$$

$$\begin{matrix}H_3C\\H_3C\end{matrix}\!\!>\overset{OH}{C}-\overset{OH}{CH}-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-$$

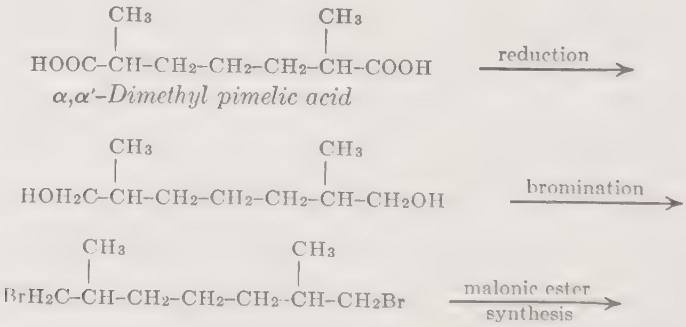
$$CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-\overset{OH}{CH}-\overset{OH}{C}\!\!<\begin{matrix}CH_3\\CH_3\end{matrix}$$

$$\Big\downarrow Pb(OCOCH_3)_4$$

$$OHC-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2-CHO$$

3,7,12,16-Tetramethyloctadecane-1,18-dialdehyde

The dialdehyde gives the corresponding acid on oxidation. Perhydronorbixin diethyl ester has been synthesized:

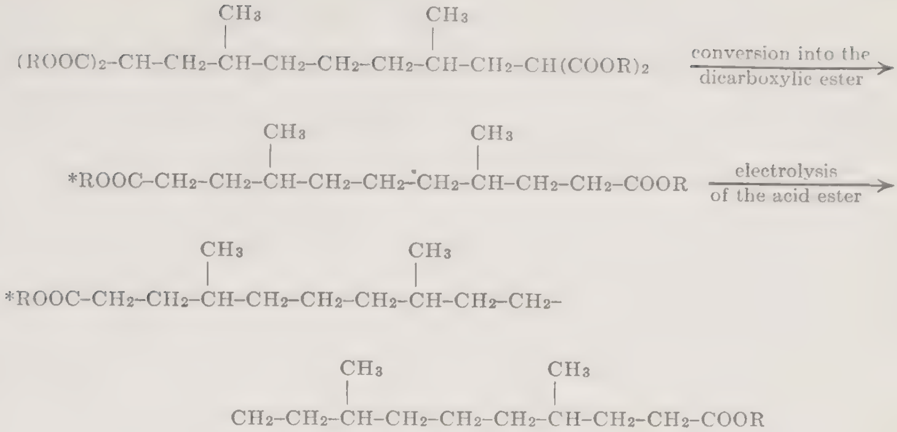


$$HOOC-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-COOH \xrightarrow{\text{reduction}}$$

α,α′-Dimethyl pimelic acid

$$HOH_2C-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2OH \xrightarrow{\text{bromination}}$$

$$BrH_2C-\overset{CH_3}{CH}-CH_2-CH_2-CH_2-\overset{CH_3}{CH}-CH_2Br \xrightarrow[\text{synthesis}]{\text{malonic ester}}$$



$$(ROOC)_2\text{-}CH\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH(COOR)_2 \xrightarrow[\text{dicarboxylic ester}]{\text{conversion into the}}$$

$$^{*}ROOC\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}\dot{C}H_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}COOR \xrightarrow[\text{of the acid ester}]{\text{electrolysis}}$$

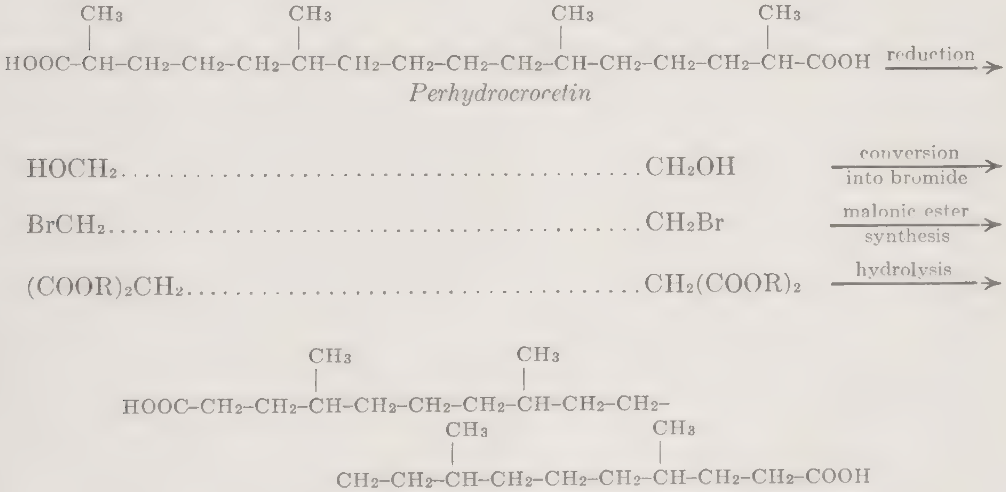
$$^{*}ROOC\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}$$

$$CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}COOR$$

Perhydronorbixin diethyl ester

(4,8,13,17-Tetramethyleicosane-1,20-dicarbethoxylate) (b. p. 207° at 0.3 mm.)

The product from natural sources is identical with that synthesized. It is remarkable that the correct configuration is obtained by synthesis as the compound contains 4 asymmetric carbon atoms and 6 inactive forms are possible. Bixane[399] above is thus 4,8,13,17-tetramethyleicosane. Finally a synthesis of perhydronorbixin[400] from perhydrocrocetin has been accomplished:



$$HOOC\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}COOH \xrightarrow{\text{reduction}}$$

Perhydrocrocetin

$$HOCH_2 \dots\dots\dots\dots CH_2OH \xrightarrow[\text{into bromide}]{\text{conversion}}$$

$$BrCH_2 \dots\dots\dots\dots CH_2Br \xrightarrow[\text{synthesis}]{\text{malonic ester}}$$

$$(COOR)_2CH_2 \dots\dots\dots\dots CH_2(COOR)_2 \xrightarrow{\text{hydrolysis}}$$

$$HOOC\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}$$

$$CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3}{CH}\text{-}CH_2\text{-}CH_2\text{-}COOH$$

Perhydronorbixin

On the other hand, perhydronorbixin has been degraded to perhydrocrocetin.[401]

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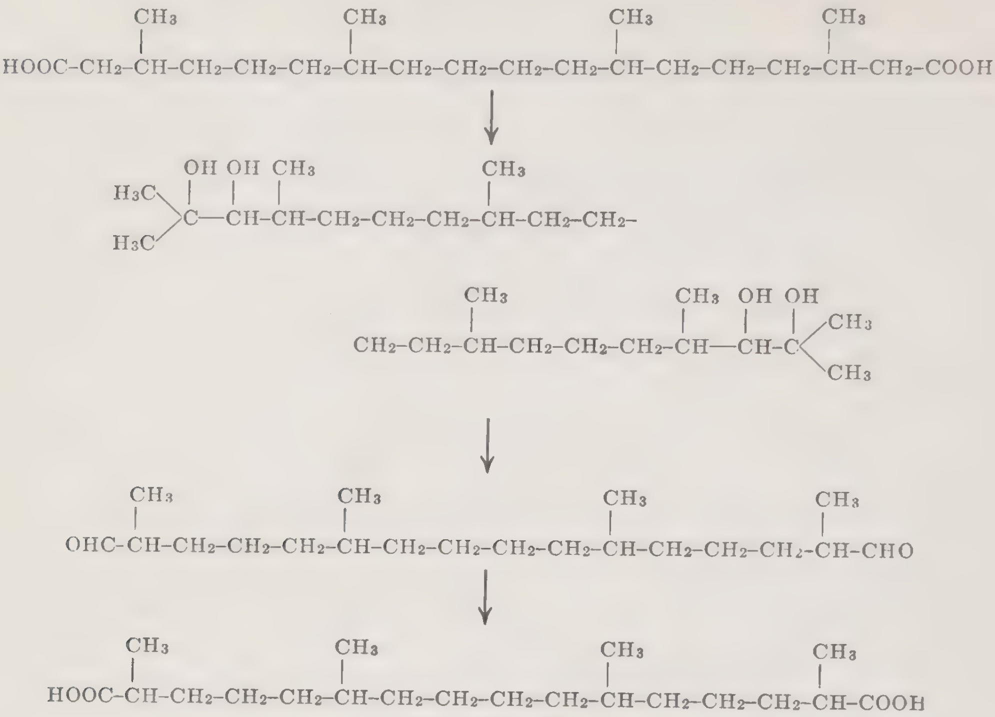
* R = C_2H_5.

[399] For a synthetic dibixane, see Karrer, Stoll, Stevens: *Helv. Chim. Acta,* **14,** 1194 (1931).

[400] Karrer, Benz: *Ibid.,* **16,** 337 (1933).

[401] Raudnitz, Peschel: *Ber.,* **66,** 901 (1933).



$$HOOC{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}COOH$$

$$\downarrow$$

$$OHC{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CHO$$

$$\downarrow$$

$$HOOC{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}CH_2{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{CH}}{-}COOH$$

Perhydrocrocetin

The degradation of lycopene to norbixin [402] has already been discussed under lycopene. Lycopene, crocetin and bixin are thus constitutionally related. An isobixin described by van Hasselt [403] is apparently not a chemical individual but merely a mixture [404] of the *cis-* and *trans-* forms of bixin. Under conditions [405] which may possibly obtain physiologically, bixin is produced by oxidation of its dihydro-compound with atmospheric oxygen.

The pigment dyes wool, cotton and silk directly to give an orange-red shade and is also used to color foodstuffs. The dyeings are fast to acid and washing but not to light. The so-called pâté de rocou [406] is the best source of bixin.

Azafrin [407] occurs in the roots and shoots of the *Scrophulariaceae Escobedia scabrifolia* and *linearis* growing in tropical America from Peru to Mexico, where it is used, under the name azafran or azafranillo, to color fats. Attention was drawn to the similarity existing between azafrin and bixin as long ago as 1911 (Liebermann). Azafrin [408]

[402] Kuhn, Grundmann: *Ber.*, **65**, 1880 (1932).

[403] van Hasselt: *Rec. trav. chim.*, **30**, 1 (1911); Karrer, Helfenstein, Widmer, van Itallie: *Helv. Chim. Acta*, **12**, 741 (1929); Kuhn, Winterstein: *Ber.*, **65**, 1873 (1932).

[404] Karrer, Takahashi: *Helv. Chim. Acta*, **16**, 287 (1933).

[405] Kuhn, Drumm: *Ber.*, **65**, 1458 (1932); Kuhn, Drumm, Hoffer, Möller: *Ber.*, **65**, 1785 (1932).

[406] Kuhn, Winterstein: *Ber.*, **65**, 1873 (1933) and p. 1877; Holmes, Bromund: *Science*, **82**, 202 (1935).

[407] Earlier work: Liebermann: *Ber.*, **44**, 850 (1911); Liebermann, Schiller: *Ber.*, **46**, 1973 (1913); Liebermann, Mühle: *Ber.*, **48**, 1653 (1915).

[408] Kuhn, Winterstein, Roth: *Ber.*, **64**, 333 (1931); **65**, 1873 (1932); Kuhn, Deutsch: *Ber.*, **66**, 883 (1933).

$(C_{27}H_{38}O_4)$[409] forms orange-red prisms [m.p. 212-214°, $[\alpha]\frac{25}{C} = -75.5°$ (alcohol), absorption bands 476-445,5-419 mμ (CS_2)] and is remarkable for its beautiful color reactions with mineral acids.

Azafrin is a carboxylic acid in which the two remaining oxygen atoms are present as hydroxyl groups (Zerewitinoff determinations); the last are tertiary hydroxyl groups, as they are not acetylated under conditions which are effective with xanthophyll. Hydrogenation reveals 7 double-linkings which, from a consideration of the absorption spectrum, are conjugated among themselves and with the carboxyl group. Toluene, *m*-xylene and *m*-toluic acid were recognized as products of thermal degradation; therefore, an arrangement assumed in earlier formulas having a methyl group in a δ-position relative to the carboxyl group can be excluded, and replaced by one with the methyl group in a γ-position, as it is in bixin. The empirical formula which this arrangement demands is further supported by determinations of the equivalent of tetradecahydroazafrin. Azafrin could thus appear to be derived from a dehydrogenated tetraterpene, of the type of carotene, by oxidative removal from one side of a C_{13} fragment (*e.g.*, ionone).

Careful oxidation with an amount of chromic acid corresponding to one atom of oxygen gave an acid, azafrinone [$C_{27}H_{36}O_4$, orange-red tablets, m.p. 191°] with absorption bands shifted toward the red end of the spectrum. Two hydrogen atoms have thus been removed, in agreement with the assumption of tertiary hydroxyl groups which could, after a ring fission, be converted into carbonyl groups. The carbonyls may be again reduced when 9 mols. of hydrogen $(7+2)$ are absorbed. This leads to the further postulate that the hydroxyl groups are adjacent, and that in fact a ring fission has taken place. Moreover, as azafrinone absorbs more in the long-wave lengths than azafrin, it may be concluded that one of the two hydroxyl groups is linked directly to the double-bonded system, so that the carbonyl group introduced enters into conjugation with it. On oxidation by Criegee's method with lead tetracetate, tetradecahydroazafrin yields a tetradecahydroazafrinone which possesses no aldehydic properties, so that the parent azafrin is a true ditertiary glycol.

Oxidation of azafrin with potassium permanganate gives geronic and α,α-dimethyl glutaric acids, so that the saturated ring system corresponds closely to that of β-carotene and must have its hydroxyl groups in positions other than those occupied in the xanthophylls, which yield no geronic acid. Destructive oxidation with chromic acid reveals three methyl groups as side-chains; all these observations are supported by the following formula:

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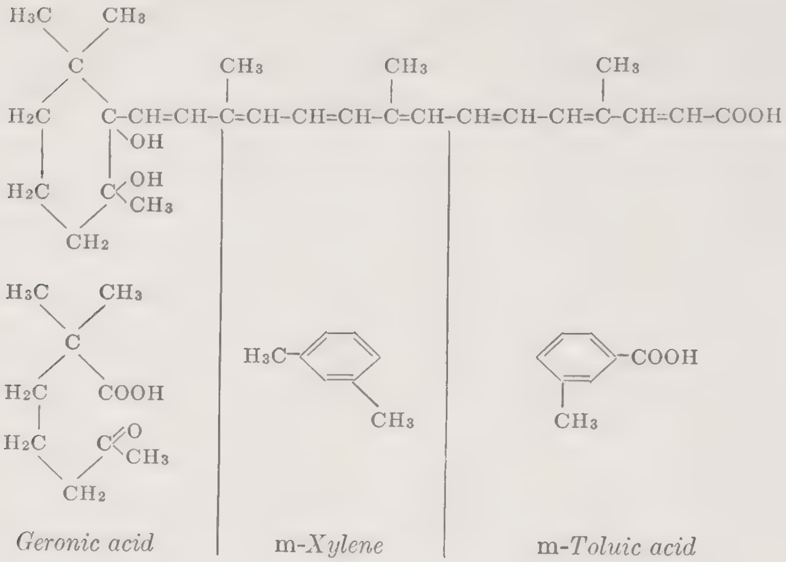
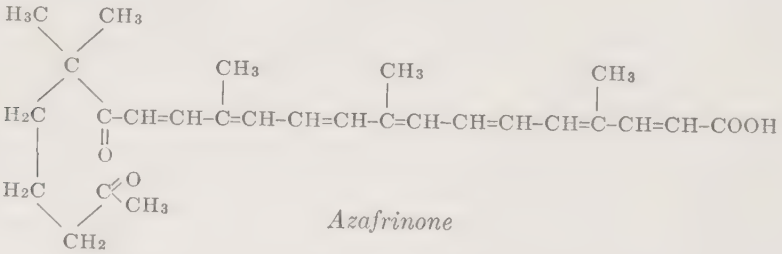
[409] For a discussion and refutation of earlier formulas, see Kuhn, Deutsch: *Ber.*, **66**, 883 (1933).



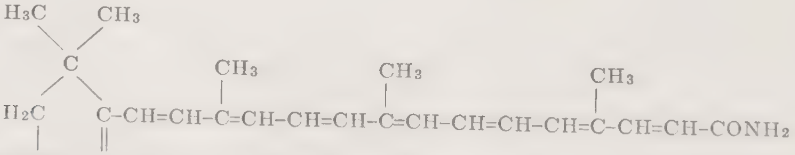
Geronic acid m-*Xylene* m-*Toluic acid*

This formula, it will be noted, is proved except for the absolute positions of the two methyl groups which appear in *m*-xylene. There can be little doubt, however, on the assumption of the formation of carotenoid carboxylic acids from the carbon skeleton deduced for lycopene, that the orientation of these methyl groups is as given previously, and this is confirmed by the following facts. On the basis of the experimental material described above azafrinone, admitting temporarily the position of the methyl groups in question, must have the constitution:



Azafrinone

Now, as outlined above under β-carotene,[410] both azafrinone and β-carotene have been converted into dehydroazafrinoneamide:



Dehydroazafrinonamide

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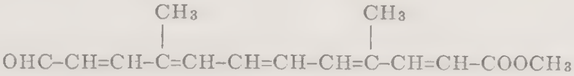
[410] Kuhn, Brockmann: *Ber.*, **67**, 885 (1934).

so that the constitution which has been proved for β-carotene provides further support for that of azafrin.

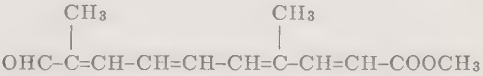
Oxidation of azafrin methyl ester gives two further degradation products [411]:

(1) The methyl ester of azafrinal I ($C_{15}H_{18}O_4$, pale-yellow needles, m.p. 160°):



$$\mathrm{OHC{-}CH{=}CH{-}\overset{\overset{\textstyle CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}CH{=}\overset{\overset{\textstyle CH_3}{|}}{C}{-}CH{=}CH{-}COOCH_3}$$

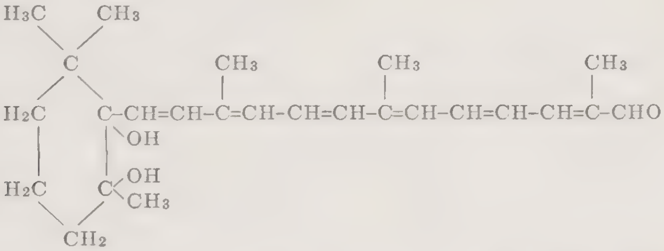
(2) The methyl ester of azafrinal II ($C_{13}H_{16}O_3$, yellow prisms, m.p. 106°):



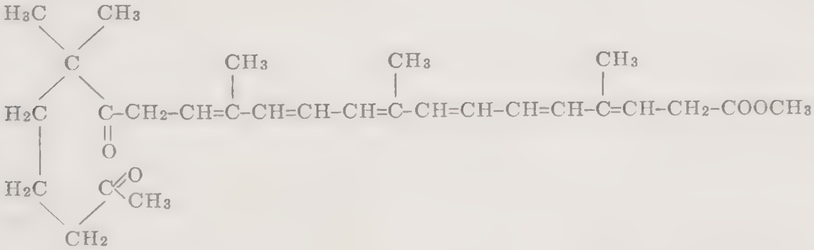
$$\mathrm{OHC{-}\overset{\overset{\textstyle CH_3}{|}}{C}{=}CH{-}CH{=}CH{-}CH{=}\overset{\overset{\textstyle CH_3}{|}}{C}{-}CH{=}CH{-}COOCH_3}$$

1,6-dimethyloctatetraene-1-al-8-carbomethoxylate

Apo-1-azafrinal results from controlled oxidation of azafrin [412] with potassium permanganate ($C_{25}H_{36}O_3$, orange-yellow needles, m.p. 171°):



The hydroxyl groups in azafrin must be in the *trans* position,[413] as is also the orientation of the double linkings.[414] Azafrinone methyl ester is reduced by zinc dust in pyridine solution to a dihydrocompound:



giving a purple-red solution with alcoholic potash which is sensitive to oxygen; on admission of air azafrinone methyl ester is reformed. Reduction of carotenoid pigments by this method affords dihydrocompounds only when the polyene chain carries carbonyl groups at each end, so that azafrin itself remains unaffected by zinc dust in pyridine solution.

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[411] Kuhn, Grundmann: *Ann.*, **516**, 95 (1935).
[412] Karrer, Ohst, Solmssen: *Helv. Chim. Acta*, **21**, 451 (1938).
[413] Kuhn, Deutsch: *Ber.*, **66**, 883 (1933) and p. 886.
[414] Kuhn, Winterstein: *Ber.*, **66**, 209 (1933) and p. 211.

Azafrin would seem to owe its color reactions to its basicity which, due in its turn to the tertiary hydroxyl group directly attached to the polyene system, is comparable with that of triphenylcarbinol; consequently in azafrinone, which lacks the hydroxyl group, these characteristic color reactions are no longer to be observed.

The rootstocks of azafrin bearing plants have excrescences which contain up to 15 per cent of the pigment. These may be extracted with acetone and the carotenoid purified by adsorption [415] on calcium carbonate. 15 kg of root yield 250 g of pigment of 90 per cent purity.

Pigments of Incompletely Determined Structure

Aphanicin [416] ($C_{80}H_{106}O_3$, red violet needles, m.p. 195°, absorption bands 533-494 mμ) is found in the blue alga *Aphanizomenon flosaquae* together with aphanin (q.v.), flavazin (epiphasic, orange-yellow, m.p. 155°) and aphanizophyll (prisms m.p. 172-173°, absorption bands, 531-494-462 mμ, possessing a hydroxyl group and forming an oxime). Aphanicin has 12 double bonds and forms an oxime. It shows vitamin A activity but only $\frac{1}{4}$ of that of β-carotene, and it is suggested that it contains two aphanin residues linked together by an oxygen bridge.

Pectenoxanthin [417] [$C_{40}H_{52}O_3$, red-brown prisms, m.p. 182°, absorption bands 518-488-454 mμ (CS_2)] occurs in the sexual glands of the mussel, *Pecten maximus,* and has been also recognized in *Pecten jarobaeus.*[418] It contains 11 ethylenic linkages and 2 hydroxyl groups (Zerewitinoff determination), and although it resembles zeaxanthin and β-carotene and possibly contains the same carbon skeleton it possesses no vitamin activity.

Spirilloxanthin,[419] $C_{48}H_{66}O_3$, is the purple pigment of *Spirillum rubrum* Esmarch. It contains 15 double linkages and probably one hydroxyl group.

Pentaxanthin [420] [$C_{40}H_{56\pm2}O_5$, red needles, m.p. 219-220°, absorption bands 506-474-444 mμ (CS_2)] was obtained by extracting sea urchins with acetone and analyzing the extract chromatographically. It resembles lutein and contains 3 hydroxyl groups.

Asteric acid [421] [$C_{40}H_{56}O_6$(?) violet powder, m.p. 185°] has been isolated from star-fish (*Asteria rubens*).

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[415] Kuhn, Deutsch: *Ber.*, **66**, 883 (1933); cf. Kuhn, Winterstein, Roth: *Ber.*, **65**, 1873 (1932).

[416] Tischer: *Z. physiol. Chem.*, **251**, 109 (1938); **260**, 257 (1939).

[417] Lederer: *Compt. rend. soc. biol.*, **116**, 150 (1934); **117**, 411 (1934); *Bull. soc. chim. biol.*, **20**, 567 (1938).

[418] Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 915 (1935).

[419] van Niel, Smith: *Arch. Mikrobiol.*, **6**, 219 (1935); French: *Science*, **88**, 60 (1938).

[420] Lederer: *Compt. rend.*, **201**, 300 (1935); *Bull. soc. chim. biol.*, **20**, 567 (1938).

[421] H. v. Euler, Hellström: *Z. physiol. Chem.*, **223**, 89 (1934); H. v. Euler, Hellström, Klussmann: **228**, 77 (1934).

Myxoxanthophyll[422] [$C_{40}H_{56\pm2}O_7$, violet needles, m.p. 169-170°, $[\alpha]_{Cd} = -255°$ (alcohol), absorption bands 518-484-450 mμ (chloroform)] is present together with myxoxanthin in the fresh-water algae *Myxophyceae, Oscillatoria rubescens.* Unlike all other known xanthophylls it is levo-rotatory.

Sulcatoxanthin[423] [$C_{40}H_{52}O_8$ (?), deep scarlet-red precipitate, m.p. 130° (indefinite), absorption bands 516-482-450 mμ (CS_2)] was obtained from the sea anemone (*Anemona sulcata*).

Flavorhodin[424] [m.p. 111°, absorption bands 503-472-441 mμ (CS_2)] is contained in the purple bacteria and is probably a hydrocarbon.

Actinioerythrin[425] [red-brown rhombohedral crystals, m.p. 83°, absorption bands 574-537-495 mμ (CS_2)] is found in the anemone *Actinia equina.* Hydrolysis yields violerythrin (dark-red crystals, m.p. 191-192°).

Cynthiaxanthin[426] [yellow prisms, m.p. 188-190°, absorption bands 517-483-452 mμ (CS_2)] accompanies astacin in *Hyacintha papillosa.*

Glycymerin[427] [m.p. 148-153°, absorption band 495 mμ (CS_2)] is present in *Pectunculus glycymeris.*

Torulin[428] [m.p. 180°, absorption bands 565-522-488 mμ (CS_2)] was isolated from red yeast (*Torula rubra*), which contains four carotenoids including β-carotene and a pigment resembling astaxanthin. Torulin is probably related to rhodoviolascin as it seems to be at the same time both epiphasic and xanthophyllic.

A pigment[429] [prisms, m.p. 195-196°, absorption bands 495 mμ (CS_2)] isolated from *Actinoloba dianthus* is probably an ester.

The pigment[429] from *Tealina felina* is also possibly an ester (Actinioerythrin ?); it is accompanied by a pigment, hydrolysis of which gave black needles [m.p. 205-208°, absorption bands 569-530-497 mμ (CS_2)].

Corallin[430] [absorption bands 509-485-465-455 mμ (ether)] occurs in *Streptothrix corallinus.*

Salmic acid[431] [deep red flaky mass, absorption bands 525-485 mμ (pyridine)] was obtained from the flesh of the salmon, which contains also carotene and xanthophyll. Two carotenoids[432] [absorption bands 505 (±5) mμ (CS_2)], isolated from the liver of the red mullet (*Regalecus*

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[422] Heilbron, Lythgoe: *J. Chem. Soc.*, **1936**, 1376.

[423] Heilbron, Jackson, Jones: *Biochemical J.*, **29**, 1384 (1935).

[424] Karrer, Solmssen: *Helv. Chim. Acta*, **18**, 1306 (1935); Karrer, Solmssen, Koenig: *Ibid.*, **21**, 454 (1938).

[425] Fabre, Lederer: *Bull. soc. chim. biol.*, **16**, 105 (1934); **20**, 567 (1938); Heilbron, Jackson, Jones: *Biochemical J.*, **29**, 1384 (1935).

[426] Lederer: *Compt. rend. soc. biol.*, **117**, 1086 (1934). The author describes also the examination of other *Ascidians*: *Bull. soc. chim. biol.*, **20**, 567 (1938).

[427] Fabre, Lederer: *Bull. soc. chim. biol.*, **16**, 105 (1934); Lederer: *Ibid.*, **20**, 567 (1938).

[428] Lederer: *Compt. rend.*, **197**, 1694 (1933); see also Fink, Zenger: *Wochenschrift Brauerei*, **51**, 89 (1934); for other sources of torulin, see Lederer: *Compt. rend. soc. biol.*, **117**, 1083 (1934).

[429] Heilbron, Jackson, Jones: *Biochemical J.*, **29**, 1384 (1935).

[430] Reader: *Ibid.*, **19**, 1039 (1925).

[431] H. v. Euler, Hellström, Malmberg: *Svensk. Kem. Tid.*, **45**, 151 (1931); Emmerie, van Eekelen, Josephy, Wolff: *Chem. Zentr.*, **1935**, II, 65; see also Bailey: *Chem. Zentr.*, **1938**, II, 707.

[432] Schmidt-Nielson, Sörensen, Trumpy: *Kong. Norsk. Vidensk. Selsk. Forhandl.*, **5**, 114, 118 (1932).

glesné) and from *Balaeptera musculus* and *Cyclopterus lumpus*, are probably identical with salmic acid.

Sarcinin[433] [absorption bands 469-440-415 mμ (petroleum)], and a xanthophyll ester,[434] [absorption bands 490-460-433 mμ (CS$_2$)], occur in *Sarcinea lutea*.

A xanthophyll[435] (absorption bands 491-458 mμ) occurs in *Bacillus lombardo pellegrini*, and another (absorption bands 506-477 mμ) is found in the Grasberger bacillus.

Pigments are found in the water-frog,[436] in the liver[437] of *Lophius piscatoris* (red) and in the sea-sponge *Microconia prolifera*.[438]

α- and β-**Bacteriorubin**[439] are coloring matters of *Bacterium halobium*. Xanthophyll-like materials occur in the mussel, *Cardium tuberlatun*,[440] and in *Ancanthodiaptomus yamanocensis*,[441] and carotenoids are recognizable in *Epiastis prolifera*[442] and in integuments[443] of insects.

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[433] Chargaff, Dieryck: *Naturwiss.*, **20,** 872 (1932); *Compt. rend.*, **197,** 946 (1933); data on the content of known carotenoids are also given.

[434] Nakamura: *Bull. Soc. Chim. Japan*, **11,** 176 (1936).

[435] Chargaff, Lederer: *Ann. Inst. Pasteur*, **54,** 383 (1935).

[436] Zechmeister, Tuzson: *Z. physiol. Chem.*, **238,** 197 (1936).

[437] Lovern, Morton: *Biochemical J.*, **25,** 1336 (1931).

[438] Bergmann, Johnson: *Z. physiol. Chem.*, **222,** 220 (1933).

[439] Petter: *Proc. Koninkl. Akad. Wetenschappen, Amsterdam*, **34,** 1417 (1931).

[440] Karrer, Solmssen: *Helv. Chim. Acta*, **18,** 915 (1935).

[441] Suginome, Ueno, Watanabe: *J. Chem. Soc. Japan*, **56,** 1199 (1935).

[442] Fox, Moe: *Chem. Zentr.*, **1938,** II, 2272.

[443] Lederer: *Compt. rend. soc. biol.*, **117,** 413 (1934).

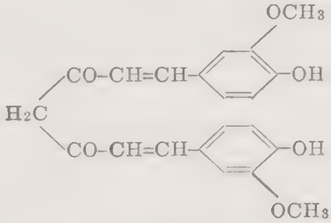
Chapter 2

Diaroylmethane Compounds

Only one natural pigment is known to belong to this group. Materials of similar structure have been prepared[1] and are of theoretical interest[2] but have not up to the present achieved technical importance.

Curcumin is a pigment occurring in the roots and shoots of *Curcuma tinctoria, longa, rotunda* and *viridiflora,*[3] which grow wild in tropical Asia and are cultivated in China, Cochin China and the East Indies. The drug obtained (yellow root, yellow ginger, terra merita, souchet, Indian saffron, turmeric) possesses an odor of ginger and a burning taste, and contains an oil[4] and a further brown coloring matter in addition to curcumin.

Jackson[5] and A. G. Perkin and Phipps[6] describe the best method of preparing curcumin from *Curcuma*[7]; their process consists in precipitating the lead salt from an alcoholic extract and further purifying the pigment. The yield is 0.65 per cent. The constitution of the dye was determined by v. Kostanecki[8] and the synthesis accomplished by Lampe.[9] Curcumin has the formula $C_{21}H_{20}O_6$ first suggested by Ciamician and Silber,[10] and forms orange-red prisms (m.p. 180-183°). It is a diferuloylmethane of the structure:



Curcumin

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[1] Ryan, Dunlea: *Proc. Roy. Irish. Acad. (B).* 32, 1 (1913); Ryan, Algar: *Ibid.*, 32, 9 (1913); Ryan, Plumkett: *Ibid.*, 32, 199 (1913); *cf.* Lampe, Godlewska: *Ber.*, 51, 1355 (1918); for a review of previous work see A. G. Perkin and A. E. Everest: "The Natural Colouring Matters," p. 392.

[2] Milobedzka, v. Kostanecki, Lampe: *Ber.*, 43, 2163 (1910).

[3] Curcumin is also found in *Curcuma aromatica* Salisb. and in *Curcuma domestica* (Temoe Lawak): Rao, Shintre: *J. Soc. Chem. Ind.*, 47, 54T (1928) and Dieterle, Kaiser: *Arch. Pharm.*, 270, 413 (1932); *cf. Tiba*, 10, 223 (1932).

[4] For the composition of this oil see: Rupe, Luksch, Steinbach: *Ber.*, 42, 2515 (1909); Rupe, Steinbach: *Ber.*, 43, 3465 (1910); 44, 584 (1911); Rupe, Wiederkehr: *Helv. Chim. Acta*, 7, 654 (1924); Rupe, Clar, St. Pfau, Plattner: *Helv. Chim. Acta*, 17, 372 (1934).

[5] Jackson: *Ber.*, 14, 485 (1881); Jackson, Menke: *Am. Chem. J.*, 4, 77 (1882).

[6] A. G. Perkin, Phipps: *J. Chem. Soc.*, 85, 63 (1904).

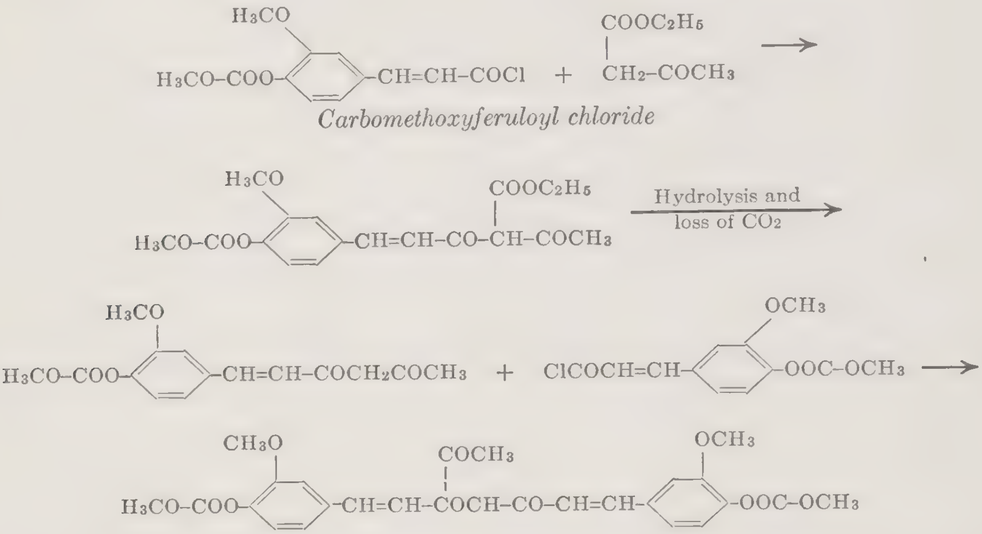
[7] Older literature: see Beilstein, Vol. 8, p. 554; Daube: *Ber.*, 3, 609 (1870) was the first to obtain the pigment in crystalline form; *cf.* also Iwanof-Gajewski: *Ber.*, 3, 624 (1870) and Kachler: *Ber.*, 3, 713 (1870).

[8] Milobedzka, v. Kostanecki, Lampe: *Ber.*, 43, 2163 (1910); Lampe, Milobedzka: *Ber.*, 46, 2235 (1913); see also Jackson, Clarke: *Ber.*, 38, 2712 (1905); 39, 2269 (1906); *Am. Chem. J.*, 45, 48 (1911).

[9] Lampe: *Ber.*, 51, 1347 (1918).

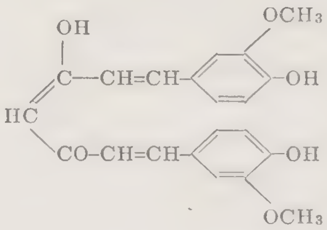
[10] Ciamician, Silber: *Ber.*, 30, 192 (1897).

It dissolves in concentrated sulfuric acid with a yellow-red coloration, and an ethereal solution shows a weak greenish fluorescence. With alkali the color of curcumin changes to red-brown (turmeric paper). The synthesis starts from carbomethoxyferuloyl chloride:


Carbomethoxyferuloyl chloride
H3CO
H3CO-COO-
-CH=CH-COCl
+
COOC2H5
CH2-COCH3
H3CO
COOC2H5
H3CO-COO-
-CH=CH-CO-CH-COCH3
Hydrolysis and loss of CO2
H3CO
H3CO-COO-
-CH=CH-COCH2COCH3
+
ClCOCH=CH-
OCH3
-OOC-OCH3
CH3O
COCH3
OCH3
H3CO-COO-
-CH=CH-COCH-CO-CH=CH-
-OOC-OCH3

On hydrolysis, the last product loses the acetyl and carbomethoxy- groups to give a product identical with natural curcumin. A further synthesis in which the units are acetylacetone and vanillin has also been effected.[11]

It is possible that natural curcumin is accompanied by minor amounts of structurally or stereo-isomeric curcumins.[12] Curcumin may assume, for example, the enolic form [13]:


OH
OCH3
C—CH=CH-
-OH
HC
CO-CH=CH-
-OH
OCH3

Degradation products of curcumin are in agreement with the constitution given above. Thus on boiling with alkali, vanillic and ferulic acids are obtained, the latter of which has been synthesized from vanillin with the aid of Perkin's reaction. Fusion with alkali yields protocatechuic acid and oxidation with permanganate yields vanillin. The action of hydroxylamine leads to the isoxazole derivative:

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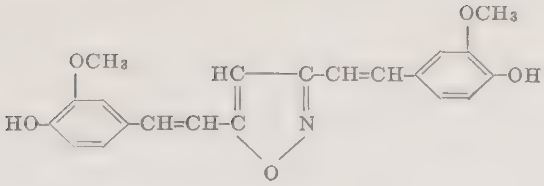
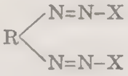
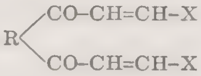
[11] Pavolini: *Chem. Zentr.*, 1938, I, 1584.
[12] Cf. the formation of two distinct diacetyl compounds: Ghosh: *J. Chem. Soc.*, 115, 292 (1919).
[13] Cf. Heller: *Ber.*, 50, 1244 (1917).



Curcumin dyes wool, silk and unmordanted cotton a yellow shade, and in spite of its poor light- and alkali-fastness is employed in China to dye silk, paper, wood and foodstuffs. The substantive properties are to be explained by the close analogy [14] with benzidine derivatives:



$$R\left\langle\begin{matrix}N{=}N{-}X\\ N{=}N{-}X\end{matrix}\right.$$



$$R\left\langle\begin{matrix}CO{-}CH{=}CH{-}X\\ CO{-}CH{=}CH{-}X\end{matrix}\right.$$

and its behavior toward mordants is attributed to its diketonic nature.[15]

On treating it with alcohol and boric acid, rubrocurcumin [16] $C_{21}H_{20}O_6$ is obtained, and this is converted on heating with dilute sulfuric acid into rosocyanin, $C_{21}H_{20}O_6$; these compounds, the preparation of which has been studied by Heller,[17] are clearly isomeric with curcumin, but their structure and indeed their homogeneity are not yet indubitably established.

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[14] Milobedzka, v. Kostanecki, Lampe: *Ber.*, **43**, 2167 (1910).

[15] Cf. Werner: *Ber.*, **41**, 1062 (1908).

[16] Clarke, Jackson: *Am. Chem. J.*, **39**, 696 (1908); earlier literature also given; the formulas given must be revised in the light of more recent work, and the whole research therefore calls for verification.

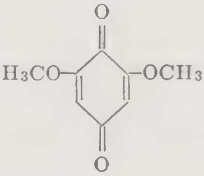
[17] Heller: *Ber.*, **47**, 887, 2998 (1914); Ryan, Dunlea: *Ber.*, **47**, 2423 (1914); Ghosh: *J. Chem. Soc.*, **115**, 292 (1919); Synthetic homologs of curcumin: Lampe, Swidersky: *Roczniki Chem.*, **18**, 120 (1938).

Chapter 3

Carbocyclic Compounds

BENZOQUINONE COMPOUNDS

Although the formation of benzoquinone derivatives in the plant may be regarded as proceeding through related phenols, the occurrence of terphenyl derivatives (see below) is particularly remarkable.

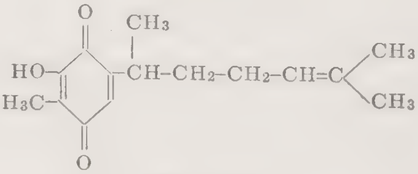


2,6-Dimethoxyquinone[1] ($C_8H_8O_4$, golden-yellow prisms, m.p. 250°) has been isolated as a by-product from *Herba adonis vernalis.*

Perezone[2] (pipitzahoic acid). This pigment is obtained in relatively large quantity from the root of *Trixis pipitzahuae* (*Perezia adnata*) (Rait del Pipitzahuae), one of the Mexican *Compositae* (yield 3.6 per cent). It has a composition corresponding to $C_{15}H_{20}O_3$ and forms orange leaflets [m.p. 102-103°, $[\alpha]_D^{20} = -17°$ (ether)]. The compound has the properties of a benzoquinone carrying a methyl group and a long side-chain. Oxidation with hydrogen peroxide yielded an unsaturated acid which was reduced to an acid, $C_9H_{18}O_2$, and which by its properties and synthesis was identified as α-ϵ-dimethylheptoic acid:

$$(CH_3)_2-CH-CH_2-CH_2-CH_2-CH(CH_3)-COOH$$

Perezone is given the following constitution:



Perezone

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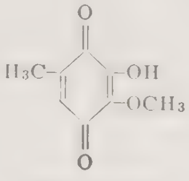
[1] W. Karrer: *Helv. Chim. Acta.* 13, 1424 (1930).

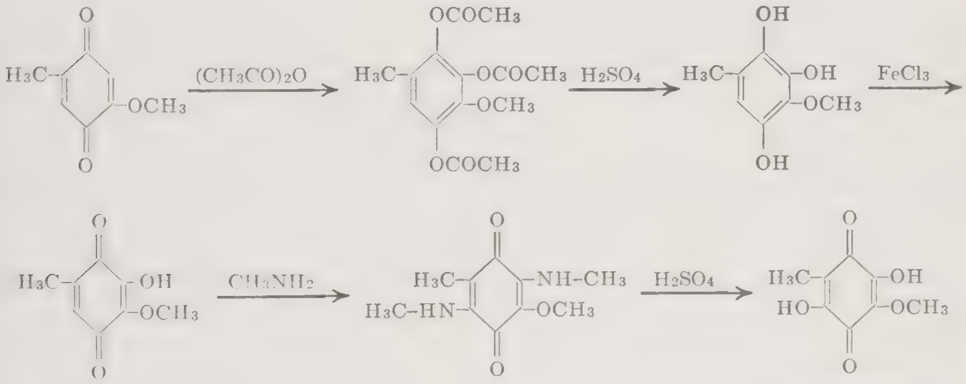
[2] Weld: *Ann.*, 95, 188 (1855); Vigener: *Sitz. Ber. Niederrh. Ges. Bonn*, 1884, 86; Mylius: *Ber.*, 18, 463 (1885); Anschütz: *Ber.*, 18, 709 (1885); Anschütz, Leather: *Ber.*, 18, 715 (1885); *Ann.*, 237, 90 (1887); Sanders: *Proc. Chem. Soc. (London)*, 22, 134 (1906); Fichter, Jetzer, Leepin: *Ann.*, 395, 1 (1912); Remfry: *J. Chem. Soc.*, 103, 1076 (1913); Kögl, Boer, *Rec. trav. chim.*, 54, 779 (1935).

Fumigatin,[3] $C_8H_8O_4$, occurs as a metabolic product together with a dihydroproduct in cultures of *Aspergillus fumigatus* growing on the Raulin-Thom medium. It forms chestnut-brown needles (m.p. 116°), and is formulated as 3-hydroxy-4-methoxy-2,5-toluquinone:



Fumagatin

Spinulosin,[4] $C_8H_8O_5$, is found in cultures of *Penicillium spinulosum* and also *Aspergillus fumigatus*, and forms plates which are almost black with a metallic lustre (m.p. 202-203°). It may be obtained after growing[5] the spores of the fungus for 14 days at 22° on Czapek-Dox glucose culture solution. The pigment was formulated as 3,6-dihydroxy-4-methoxy-2,5-toluquinone[6] and this structure was recently confirmed by synthesis:



Spinulosin

Polyporic acid. A fungus of the family Polyporeae found growing on diseased oak-trees contains a coloring principle which is readily recognized by its deep-violet solution in dilute ammonia. The first investigation on polyporic acid was carried out and the name suggested by Stahlschmidt[7]; the constitution was elucidated by Kögl[8] using the original preparation of Stahlschmidt and also material obtained from *Polyporus nidulans*

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[3] Anslow, Raistrick: *Biochemical J.*, 32, 687, 2288 (1938); Anslow, Ashley, Raistrick: *J. Chem. Soc.*, **1938,** 439.

[4] Anslow, Raistrick: *Biochemical J.*, 32, 687, 2288 (1938); Anslow, Ashley, Raistrick: *J. Chem. Soc.*, 1938, 439; Birkinshaw, Raistrick: *Phil. Trans. Roy. Soc. London (B)*, 220, 245 (1931).

[5] Preparation, see also Kögl in Klein, "Handbuch der Pflanzenanalyse," **III,** 2, p. 1421.

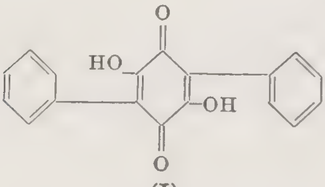
[6] Anslow, Raistrick: *Biochemical J.*, 32, 803 (1938).

[7] Stahlschmidt: *Ann.*, 187, 177 (1877); 195, 365 (1879).

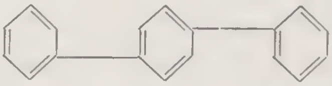
[8] Kögl: *Ann.*, **447,** 78 (1926); Kögl, Becker: *Ann.*, **465,** 219 (1928).

(Pers.)[9]; the color reaction has also been observed in *Polyporus rutilans* (P).

Polyporic acid, $C_{18}H_{12}O_4$, forms glistening violet-brown leaflets and yields a diacetyl derivative (yellow needles, m.p. 209°). The pigment has proved to be identical with 3,6-dihydroxy-2,5-diphenyl-1,4-benzoquinone[10] (I) which agrees with the previous observation of the formation of terphenyl (II) on distillation with zinc dust:

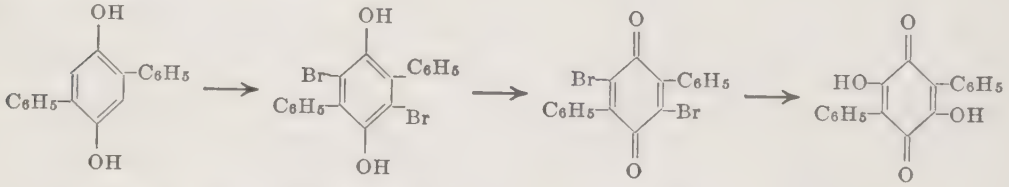


(I)

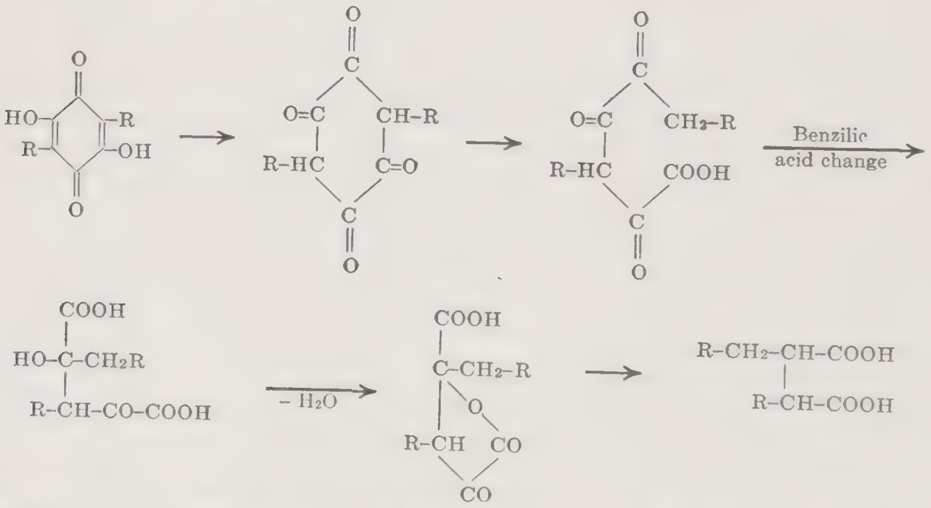


(II)

The preparation of polyporic acid by the successive bromination, oxidation and hydrolysis of diphenylhydroquinone has been effected by Adams and Shildneck:[11]


OH
C6H5
C6H5
OH
OH
Br
C6H5
C6H5
Br
OH
O
Br
C6H5
C6H5
Br
O
O
HO
C6H5
C6H5
OH
O

According to Fichter[12] fission of dialkyldihydroxyquinones gives dialkylated succinic acids:


O
HO
R
R
OH
O
O
C
O=C
CH-R
R-HC
C=O
C
O
O
C
O=C
CH2-R
R-HC
COOH
C
O
Benzilic acid change
COOH
HO-C-CH2R
R-CH-CO-COOH
- H2O
COOH
C-CH2-R
O
R-CH
CO
CO
R-CH2-CH-COOH
R-CH-COOH

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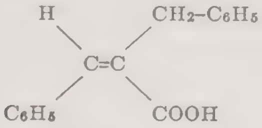
[9] Klingemann: *Ann.*, 275, 89 (1893); he was unable to detect the pigment in fungi growing where it had originally been found in the Eschweiler forest; see also Bamberger, Landsiedl: *Monatsh.*, 30, 673 (1909).

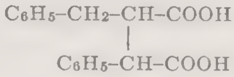
[10] Fichter: *Ann.*, 361, 363 (1908).

[11] Shildneck, Adams: *J. Am. Chem. Soc.*, 53, 2373 (1931); cf. Pummerer, Prell., *Ber.*, 55, 3105 (1922).

[12] Fichter: *Ann.*, 361, 363 (1908); Kögl, Becker: *Ann.*, 465, 211 (1928), particularly p. 219.

Two stereoisomeric doubly substituted succinic acids thus arise. When polyporic acid was subjected to this fission, 3 acids, of which two were isomeric α-benzylcinnamic (I) and one phenylbenzylsuccinic acid (II) were obtained:


(I)



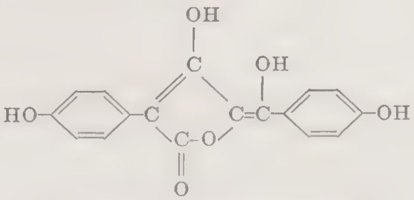
$$\begin{array}{l} C_6H_5{-}CH_2{-}CH{-}COOH \\ \qquad\qquad\quad | \\ \quad C_6H_5{-}CH{-}COOH \end{array}$$

(II)

Thus not only carbon dioxide but also to some extent oxalic acid has been split off. Lack of material made it impossible to isolate the phenylbenzylsuccinic acid.

Isolation: Polyporus nidulans is extracted with dilute ammonia, the violet solution precipitated with hydrochloric acid and the pigment purified by conversion into its potassium salt. The abundance of pigment in the fungus is remarkable and a yield of 18 per cent of the weight of fungus is obtained.

Atromentin. This pigment was found by Thörner [13] in *Paxillus atrotomentosus Batsch,* a fungus which frequently appears on old tree trunks in fir woods. Kögl [14] has improved its preparation and determined its constitution; there is no indication that it occurs as a glycoside. Atromentin, $C_{18}H_{12}O_6$, forms shining leaflets with a bronze or chocolate-brown sheen, possesses no melting point and is sublimed only with great difficulty. It contains 4 hydroxyl groups (the tetraacetyl derivative forms yellow leaflets, m.p. 245°) and its quinonoid nature is revealed by the formation of a hexamethylleucoatromentin (colorless needles, m.p. 238°). Reduction with zinc dust gives terphenyl, and oxidation with hydrogen peroxide gives *p*-hydroxybenzoic acid, although in alkaline solution the lactone of atromentic acid (derived from the red atromentic acid, a *p,p'*-dihydroxypulvic acid) is obtained:



Lactone of atromentic acid

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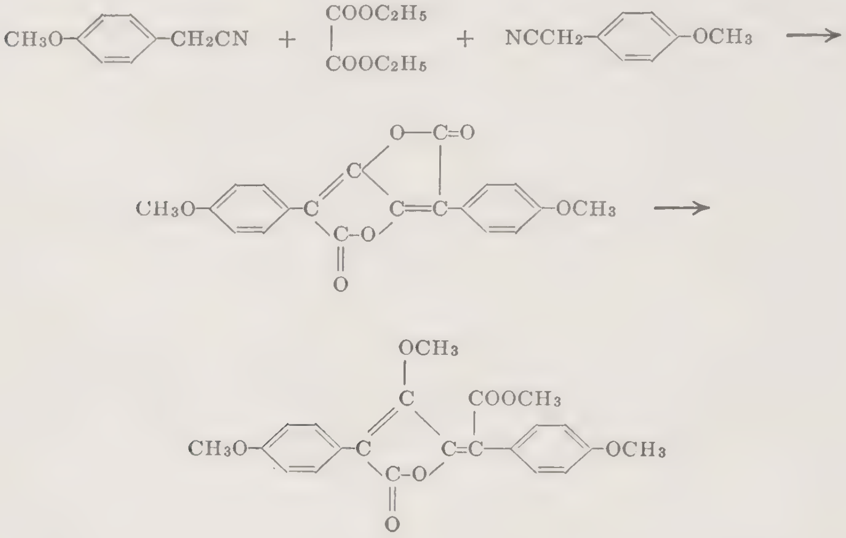
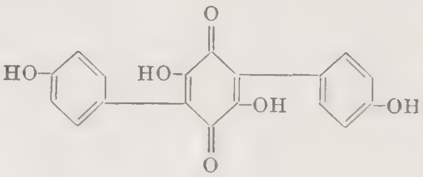
[13] Thörner: *Ber.*, **11**, 533 (1878); **12**, 1630 (1879).

[14] Kögl, Postowsky: *Ann.*, **440**, 19 (1924); **445**, 159 (1925); Kögl, Becker: *Ann.*, **465**, 211 (1928); Kögl: *Ann.*, **465**, 243 (1928).

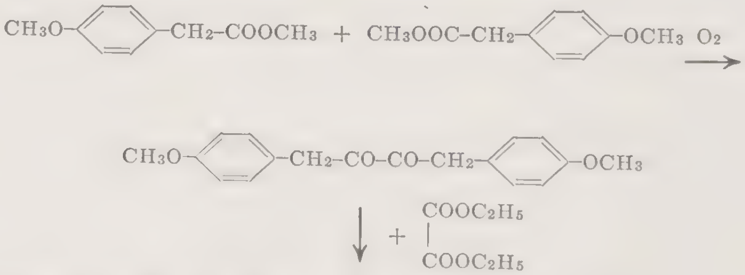
On more drastic fission with alkali the latter splits into oxalic acid and *p*-hydroxyphenylacetic acid. A synthesis[15] of the tetramethyl derivative of the above lactone based on Volhard's synthesis was effected in the following steps:



Atromentin was thus constituted as a 2,5-di-(*p*-hydroxyphenyl)-3,6-dihydroxy-1,4-benzoquinone:



and a synthesis of this structure was accomplished by the method of preparing dihydroxyquinones by Fichter, and was later improved by Kögl[16]:



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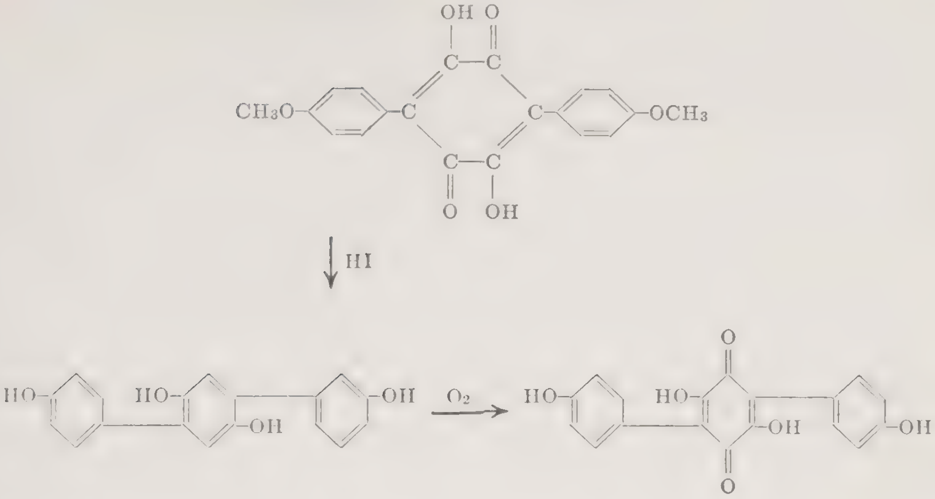
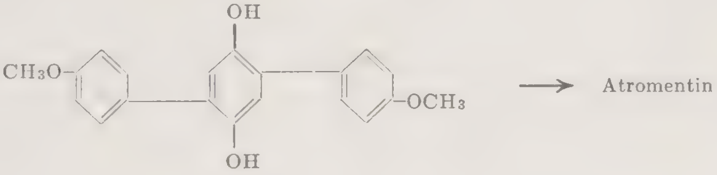
[15] Kögl, Becker: *Ann.*, **465**, 243 (1928); *cf.* Volhard: *Ann.*, **282**, 1 (1894).
[16] Kögl, Becker: *Ann.*, **465**, 243 (1928).

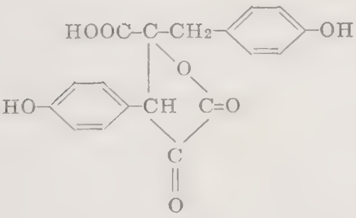


Shildneck and Adams[17] have also obtained atromentin by successive bromination, oxidation and hydrolysis of a dianisylhydroquinone (I) prepared by Pummerer and Prell[18]:

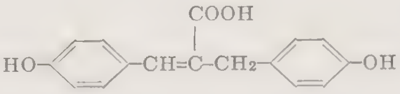


(I)

The fission of atromentin by alkali proceeds in a manner similar to that mentioned under polyporic acid (see above). The γ-carboxylic acid of α-keto-β,γ-di(p-hydroxyphenyl)-butyrolactone (II)



(II)



(III)

is obtained together with two isomeric compounds which must be formulated as p-hydroxy-α-(p′-hydroxybenzyl)-cinnamic acids (III). Atromentin dyes wool a dull-brown shade and imparts a greenish tint to chromium-mordanted material.

Isolation: The dried fungus is extracted with sodium hydroxide and the red-brown filtrate precipitated with hydrochloric acid. Yield, 1.5-2 per cent of the air-dried material.

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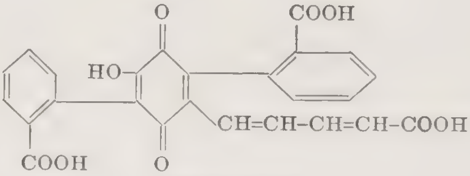
[17] Shildneck, Adams: *J. Am. Chem. Soc.*, **53**, 2373 (1931).
[18] Pummerer, Prell: *Ber.*, **55**, 3105 (1922).

Muscarufin. The pigment of the toadstool (*Amanita muscaria* L.) was first described by Griffiths,[19] later by Zellner[20] and its purification and constitution were studied recently by Kögl and Erxleben,[21] who assigned the name. The coloring matter, which probably occurs as a glycoside, has a composition corresponding to $C_{25}H_{16}O_9$ and forms orange-red rhombic crystals (m.p. 275.5°). The parent pigment gives a mono-acetyl derivative (orange-yellow needles, m.p. 197°) but reductive acetylation yields a triacetyl compound as colorless needles (m.p. 184°). Destructive distillation with zinc dust gives terphenyl; and the formation of a hexahydromuscarufin on catalytic reduction indicates that two double linkings must be present, in addition to the quinonoid system. Three carboxyl groups are revealed by electrometric titration; and, as oxidation with alkaline hydrogen peroxide yielded phthalic acid whereas the hydrogenated muscarufin gave adipic acid, it was concluded that (a) the phenolic hydroxyl group in muscarufin must be placed in position 3 or 6 of the quinone ring, and (b) that two of the carboxyl groups must be in 2′ and 2″ positions, while the second carboxyl group which appears in phthalic acid originates as carbon atom 2 or 5 of the quinone ring. Finally, the presence of a doubly unsaturated carboxylated side-chain was confirmed by the condensation of triacetyl-leucomuscarufin with maleic anhydride, so that the following constitution represents the reactions of muscarufin:



Muscarufin

Stereoisomerism of the type discovered by Kenner[22] among *o*-substituted diphenic acids due to restricted rotation might be expected in a double diphenyl system of the type shown above, wherein each diphenyl system carries 3 *o*-substituents, but this expectation has not yet been experimentally verified.

Isolation: The reddish skins of the toadstools are allowed to stand in ethyl alcohol at 0°. The extract is then precipitated with silver nitrate, the silver salt decomposed and the pigment further purified.

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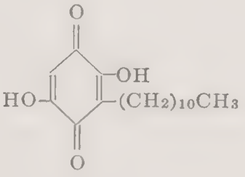
[19] Griffiths: *Compt. rend.*, 122, 1342 (1896); 130, 42 (1900).

[20] Zellner: *Monatsh.*, 27, 282 (1906).

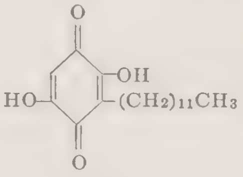
[21] Kögl, Erxleben: *Ann.*, 479, 11 (1930).

[22] Christie, Kenner: *J. Chem. Soc.*, 121, 614 (1922); *cf.* Kuhn, Albrecht: *Ann.*, 464, 91 (1928); Kuhn, Goldfinger: *Ann.*, 470, 183 (1929).

Embelin.[23] This pigment, formerly termed elbelic acid, has a composition corresponding to $C_{17}H_{26}O_4$[25a] or $C_{18}H_{28}O_4$[23a] (golden yellow platelets or needles, m.p. 142°), and occurs in the berries of the Indian shrub *Embelia ribes*, and also in *Myrsine africana Linn*,[24] an Indian shrub of the same family. Embelin yields *n*-lauric acid on oxidation; it combines with two aldehyde residues, and also forms either a di- or tetra-oxime. Compounds corresponding to both formulas have been synthesized.[25a] Neither of the substances shows a melting point depression with natural material; but the leuco acetate from the natural substance shows a melting point depression (8°) with the leuco acetate of formula II but not of formula I. Embelin dyes silk and wool from alcoholic solution, and possesses an anthelmintic action.

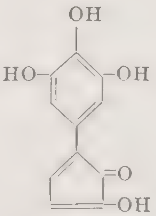


I



II

Dryophantin[26] ($C_{23}H_{28}O_{15}$, dark-red needles, m.p. 220°) occurs in Red-Pea galls on the leaves of varieties of oak (*Quercus peduncalata* and *Dryophanta divisa*). The pigment is a glyocoside and may be degraded to give 2 mols. of dextrose and 1 mol. of purpurogallin:



Dryophantin

This is the first occurrence of purpurogallin in nature to be noted. The pigments of plant-galls have also been termed gallorubrones.

Phoenicin[27] ($C_{14}H_{10}O_6$, red tablets, m.p. 230-231°) was isolated from a culture of *Penicillium phoeniceum*, 100 cc. of culture solution containing

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[23] Warden: *Pharm. J.*, (third series), **18**, 601 (1887-1888); **19**, 305 (1888-1889); Heffter, Feuerstein: *Arch. Pharm.*, 238, 15 (1900); Kaul, Ray, Dutt: *J. Indian Chem. Soc.*, **6**, 577 (1929).

[23a] Hasau, Stedman: *J. Chem. Soc.*, 2112 (1931).

[24] Krishna, Varma: *J. Indian Chem. Soc.*, **13**, 115 (1936).

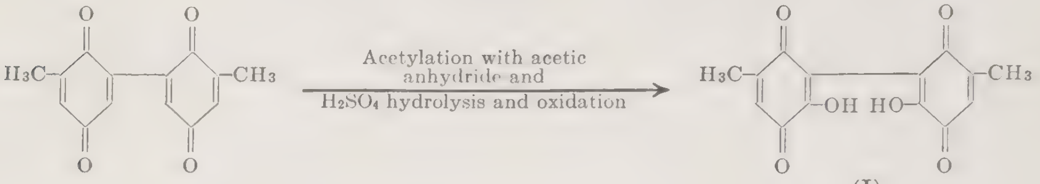
[25] Paranjpé, Gokhalé: *Chem. Zentr.*, **1933**, I, 3215.

[25a] Asano, Yamaguti, *J. Pharm. Soc. (Japan)*, **60**, 105 (1940); Asano, Hase: *Ibid.*, **60**, 650 (1940).

[26] Nierenstein: *J. Chem. Soc.*, **115**, 1328 (1919).

[27] Posternak: *Helv. Chim. Acta*, **21**, 1326 (1938); Friedheim: *Ibid.*, **21**, 1464 (1938).

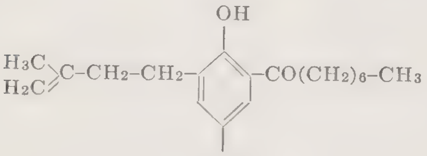
3.5 g of mycelium, yielding 44 mg of pigment. The constitution (I) follows from its properties and synthesis:


Acetylation with acetic anhydride and
H2SO4 hydrolysis and oxidation

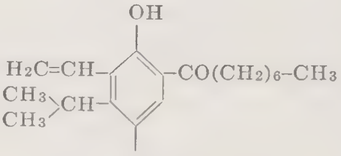
(I)

The following coloring matters were isolated during an examination of 25 strains of the *Aspergillus glaucus* series (see also Rubroglaucin).

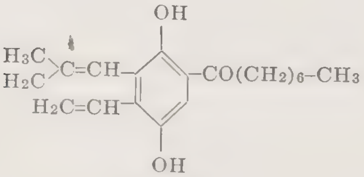
Flavoglaucin[28] ($C_{19}H_{28}O_3$, lemon-yellow needles, m.p. 104°) is probably 2-isopentenyl-, 2-vinyl-3-isopropyl- or 3-vinyl-2-isopropyl-6-*n*-octoylhydroquinone (I, II or III):



(I)



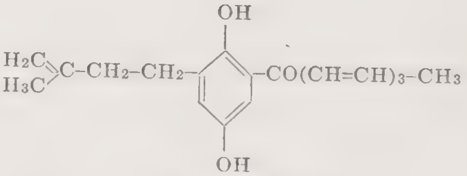
(II)



(III)

Flavoglaucin

Auroglaucin[28] ($C_{19}H_{22}O_3$, orange-red needles, m.p. 153°) is given the constitution:



Auroglaucin

or a similar one corresponding to formula (II) or (III) for flavoglaucin above.

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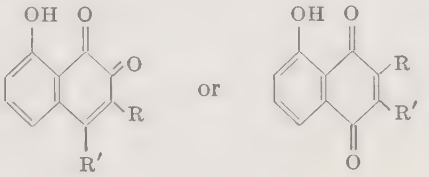
[28] Gould, Raistrick: *Biochemical J.*, 28, 1640 (1935); Raistrick, Robinson, Todd: *J. Chem. Soc.*, 1937, 80; Cruickshank, Raistrick, Robinson: *J. Chem. Soc.*, 1938, 2056; Cruickshank, Robinson: *J. Chem. Soc.*, 1938, 2064.

NAPHTHOQUINONE COMPOUNDS

The formation of naphthoquinone derivatives in the plant may be regarded as proceeding from benzoquinones by a condensation similar to that of the Diels-Alder synthesis. Napthoquinones have recently received attention following the discovery that a number of them exhibit vitamin K activity (*v. infra*).

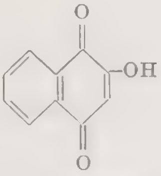
There seems to be only one member of this class of natural pigments which may be included among the true β-naphthoquinones. Dunnione (q.v.) is a β-naphthoquinone containing an additional hetero-ring. *Celastrol,* $C_{22}H_{30}O_3$, the coloring matter of the outer bark of *Celastrus scandeus,* contains one hydroxyl group and a quinone system. It is formulated as a naphthoquinone:



$R + R' = C_{12}H_{26}$

as it yields 3-hydroxyphthalic acid on oxidation with permanganate; all its reactions, however, do not seem to be easily reconciled with this formulation. The mono-methyl ether shows some vitamin K activity, but this is not shared by celastrol itself.[28a]

Lawsone. A pigment lawsone[29] (henna) ($C_{10}H_6O_3$, yellow crystals, m.p. 192-195°) occurs in the leaves of *Lawsonia inermis* L. and also in *Lawsonia alba* Lam., the Indian ornamental garden or hedge shrub Mehedi from which it is extracted by aqueous sodium carbonate. It dyes wool and silk an orange shade and is identical with 2-hydroxy-1,4-naphthoquinone.



Lawsone

Juglone[30] (nucin, regianin) is widely distributed in the *Juglandeae* family and occurs as an α-hydrojuglone[31] in the shell of the walnut.

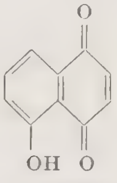
[28a] Gisvold, *J. Am. Pharm. Soc.*, 28, 440 (1939); 29, 12 (1940).

[29] Tommasi: *Gazz. chim. ital.*, 50, I, 263 (1920); Lal, Dutt: *J. Indian Chem. Soc.*, 10, 577 (1933); Candelli: *Boll. chim. farm.*, 13, 85 (1934); Cox: *Analyst*, 63, 397 (1938).

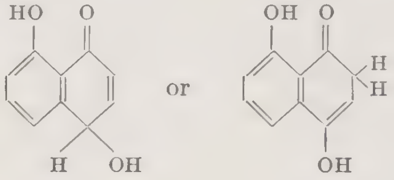
[30] Older literature: Beilstein, Bd. 8, p. 308; V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," II, 2, 392; microchemical detection: Tunmann, *Pharm. Zentralhalle*, 53, 1005 (1912); R. Fischer, Stauder: *Ibid.*, 72, 97 (1931); complex salts: Ciusa: *Ann. chim. appl.*, 16, 127 (1926); Mangini: *Gazz. chim. ital.*, 61, 820 (1931).

[31] Willstätter, Wheeler: *Ber.*, 47, 2796 (1914).

Juglone, $C_{10}H_6O_3$, forms yellow-red needles or prisms (m.p. 153-154°) and is 5-hydroxy-1,4-naphthoquinone. The constitution of juglone is indicated by its formation by oxidizing 1,5-dihydroxynaphthalene and by its oxidation to 3-hydroxyphthalic acid. Its precursor in the plant, α-hydrojuglone, is 1,4,5-trihydroxynaphthalene, which may exist in the keto form β-hydrojuglone:

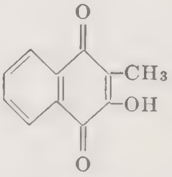


Juglone



It is isolated from the nut-shells by extraction with ether.[32] Juglone dyes wool which is mordanted with aluminum, chromium or iron salts a brownish-yellow color, and cotton mordanted with aluminum salts a rose tint.

Phthiocol,[33, 34] the pigment of human tubercular bacillae, has a composition corresponding to $C_{11}H_8O_3$, crystallizes in yellow prisms (m.p. 173-174°) and is 2-methyl-3-hydroxy-1,4-naphthoquinone. It was prepared from strains grown on Long synthetic culture medium and first synthesized from 2-methyl-1,4-diacetoxynaphthalene.[35] Phthiocol exhibits the activity of vitamin K.



Phthiocol

Plumbagin[36] [$C_{11}H_8O_3$, golden-yellow silky needles, m.p. 77-78°] occurs in Chitraka or Chita, the root of an Indian shrub *Plumbago rosea* (also in *Plumbago zeylanica* and *Plumbago europaea*) which is valued for its medicinal properties. Plumbagin is 5-hydroxy-2-methyl-1,4-naphthoquinone[37] and was synthesized in the following steps[38]:

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[32] Bernthsen, Semper: *Ber.*, **18**, 205 (1885).

[33] Anderson, Newman: *J. Biol. Chem.*, **101**, 773 (1933).

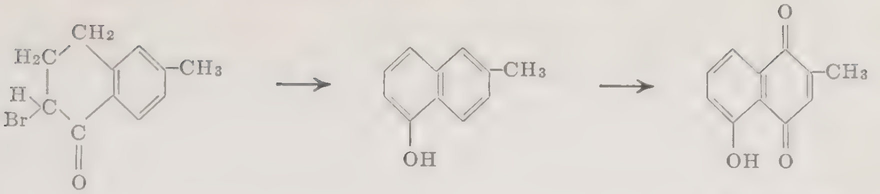
[34] Anderson, Newman: *Ibid.*, **103**, 197 (1933). Synthesis of phthiocol: Anderson, Creighton, *J. biol. Chem.*, **130**, 429 (1939).

[35] Anderson, Newmann: *Ibid.*, **103**, 405 (1933); U. S. P. 1984491. (New Haven Dispensary) *Chem. Zentr.*, **1935**, II, 1613; Hooker: *J. Am. Chem. Soc.*, **58**, 1174 (1936).

[36] Roy, Dutt: *J. Indian Chem. Soc.*, **5**, 419 (1928); Madinaveitia, Gallego: *Anales soc. espan. fis. quim.*, **26**, 263 (1928); Katti, Patwardhan: *J. Indian Inst. Sci.*, **A15**, 9 (1932).

[37] Madinaveitia, de Buruaga: *Anales soc. espan. fis. quim.*, **27**, 647 (1929); de Buruaga: *Ibid.*, **31**, 185 (1933).

[38] Fieser, Dunn: *J. Am. Chem. Soc.*, **58**, 572 (1936); pharmacological action, Bhatia, Lal: *Chem. Zentr.*, **1933**, I, 2720.

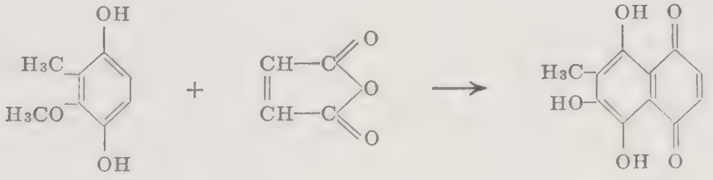


Plumbagin

The pigment originally termed droserone from Drosera rotundifolia is identical [39] with plumbagin.

Droserone.[40] Two red pigments, droserone and hydroxydroserone (see below) are found between the inner part and the outer covering of the bulbous root growths of *Drosera Whittakeri,* a plant flourishing in Australia in the district around Adelaide. Droserone, $C_{11}H_8O_4$, forms pale-yellow needles (m.p. 181°) and yields a diacetyl derivative (m.p. 109°) and is probably 3,5(8?-)-dihydroxy-2-methyl-1,4-naphthoquinone.[41]

Hydroxydroserone,[42] the second pigment of *Drosera,* has the empirical formula $C_{11}H_8O_5$, crystallizes in red plates (m.p. 192-193°) and forms a triacetyl derivative as yellow crystals (m.p. 153-154°). Its constitution as trihydroxymethylnaphthoquinone was suggested as long ago as 1893 by Rennie, and its orientation as 3,5,8-trihydroxy-2-methyl-1,4-naphthoquinone (I) was deduced from its absorption spectrum and confirmed by the following elegant synthesis [43]:



(I)

Echinochrome.[44] This term describes a number of pigments occurring in *Arbacia* and *Paracentiosus* (sea urchins) which are distinguished, according to Kuhn, by the following properties: *Echinochrome A* is the pigment first found by Lederer [45] in the ova of *Arbacia aequituberculata* and *Arbacia pustulosa;* it forms deep-red needles [m.p. 220°, absorption bands 531-495-461 mμ (CS_2)] and has the empirical formula $C_{12}H_{10}O_7$. *Echinochrome* P [m.p. 229° (decomp.), absorption bands 555-515-465 mμ (CS_2)] is found in *Arbacia pustulosa. Spinochrome P* [$C_{12}H_{10}O_8$, m.p. 185°, absorption bands 595-548-507 mμ (CS_2)] was isolated from *Strongylocentrotus.*

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[39] Witanowski: *Chem. Zentr.,* **1935,** I, 1069; Dieterle: *Arch. Pharm.,* **273,** 235 (1935); Dieterle, Kruta: *Ibid.,* 274, 457 (1936).

[40] Rennie: *J. Chem. Soc.,* **51,** 371 (1887); **63,** 1083 (1893); Lugg, Macbeth, Winzor: *Ibid.,* **1937,** 1597.

[41] Macbeth, Winzor: *Ibid.,* **1935,** 336; reduction potential: Lugg, Macbeth, Winzor: *Ibid.,* **1936,** 1457.

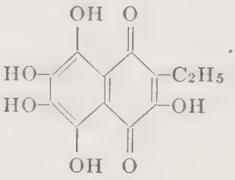
[42] Rennie: *Ibid.,* **51,** 371 (1887); **63,** 1083 (1893); Macbeth, Price, Winzor: *Ibid.,* **1935,** 325; Macbeth, Winzor: *Ibid.,* **1935,** 334; Lugg, Macbeth, Winzor: *Ibid.,* **1936,** 1457.

[43] Winzor: *Ibid.,* **1935,** 336; cf. Kuroda, Wada: *Sci. Papers Inst. Phys. Chem. Research (Tokyo),* **34,** 1740 (1938); for data suggesting the occurrence of naphthoquinone derivatives in other *Drosera* species, see Fünstück: *Ber. deut. bot. Ges.,* **34,** 160 (1916); Sabalitschka: *Südd. Apoth. Zt.,* **61,** 183 (1921); *Arch. Pharm.,* **261,** 217 (1923); Dieterle: *Arch. Pharm.,* **260,** 45 (1923).

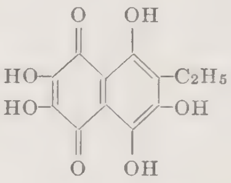
[44] Kuhn, Wallenfels: *Ber.,* **72,** 1407 (1939).

[45] Lederer, Glaser: *Compt. rend.,* **207,** 454 (1938).

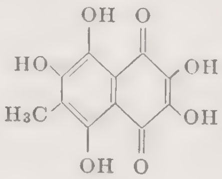
Echinochrome A yielded naphthalene on distillation with zinc dust, thus accounting for ten of the carbon atoms, the remaining two being present as an ethyl group, as propionic acid is obtained on oxidation with chromic acid. Moreover acetylation revealed the presence of 7 hydroxyl groups, and it was therefore concluded that the pigment is the quinone derived from 1,3,4,5,6,7,8-heptahydroxy-2-ethylnaphthalene:



or



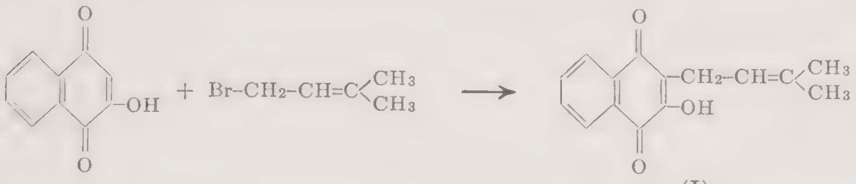
a conclusion confirmed by comparative color reactions. Echinochrome A is the chemotactic principle ejected by the eggs into the sea-water surrounding it and induces mobility in the spermatozoa. The more exact chemistry of these pigments awaits clarification. Some workers [45a] have distinguished between echinochrome and spinochrome; an isoechinochrome was also obtained, m.p. 247°. Spinochrome was given the formula $C_{12}H_{10}O_8$. Other workers, however, favor the formula $C_{12}H_{10}O_7$, and regard spinochrome as an isomeride rather than an oxygenated derivative of echinochrome; and a more detailed examination appeared to confirm this view.[45b] Still others have isolated what appear to be a number of individual polyhydroxynaphthoquinones from *Pseudocentrotus, Heterocentrotus* and other species.[45c] These latest workers obtained spinochrome *aka* (m.p. 285-295°), spinochrome F (m.p. 229°), and spinochrome M (m.p. 193°). Of these the first was believed to have the structure:



and a number of similar compounds were synthesized for comparative purposes.

[45a] Lederer, Glaser, *Compt. rend.*, **208**, 1939 (1939).
[45b] Musajo, Minchilli, *Ricerca sci.*, **11**, 187 (1940); *Gazz. chim. ital.*, **70**, 287 (1940).
[45c] Kuroda, Ohshima, *Proc. Imp. Acad. Tokyo*, **16**, 214 (1940).

Lapachol is found in the Taigu (or lapachol) wood obtained from a number of South American *Bignoniaceae*, in bethabarra wood from the west coast of Africa and in Surinam "greenheart" wood.[46] It has the formula $C_{15}H_{14}O_3$, crystallizes in yellow prisms (m.p. 139.5-140.5°) and dissolves in alkali or alkali carbonate to give a red solution. Lapachol contains one hydroxyl group, and as it yields naphthalene and isobutylene on distillation with zinc dust, it contains a naphthalene nucleus carrying a C_5 side-chain. The constitution (I), 2-(γ,γ-dimethylallyl)-3-hydroxy-1,4-naphthoquinone is verified by its synthesis from 2-hydroxy-1,4-naphthoquinone and γ,γ-dimethylallylbromide:



(I)

when the normal allylation product, 2-(γ,γ-dimethylallyloxy)-1,4-naphthoquinone is also obtained. The possibility that the last is an intermediate from which lapachol is formed by a Claisen rearrangement, in which case the constitution above would have to be modified, has been shown by model experiments to be unlikely.[47] The dye is obtained by extracting the wood with aqueous alkali carbonate. Both lapachol and its methyl ether show the activity of vitamin K.

Tecomin.[48] This is a mixture of pigments found in the wood of *Bignonia tecoma* (Ipé-tabacco wood), a tree growing in Brazil, and in similar species, *e.g.*, *Tecoma ipé* and *Tecoma ochrana.* The material extracted from the wood by alcohol may be separated, by treatment with sodium carbonate solution, into a soluble constituent which is identical with lapachol, and an insoluble compound crystallizing in pale yellow needles (m.p. 242°) of unknown constitution. A calcareous infusion of the wood shavings is used as a native dye for cotton.

Lomatiol[49] is the coloring principle of the seeds of *Lomatia ilicifolia* and *longifolia* (*Proteaceae* family) growing in Australia (N. S. Wales and Victoria) and is also found in other Australian, but not in Chilean, species. It has the formula $C_{15}H_4O_4$, and crystallizes in yellow needles (m.p. 127°). Earlier views on the constitution of lomatiol were based on its conversion into hydroxy-β-lapachone (I) by the prolonged action of concentrated sulfuric acid; and more recent work[50] has shown that on hydrogenation lomatiol gives hydrolomatiol (II) and hydrolapachol (III), the latter also obtained from lapachol:

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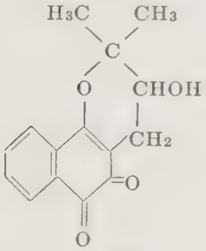
[46] Older literature: V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," II, 2, p. 395; Oesterle: *Arch. Pharm.*, **251**, 301 (1913); **254**, 346 (1916); Hooker: *J. Am. Chem. Soc.*, **58**, 9163, 1168, 1174, 1181, 1190 (1936); Hooker, Steyermark: *Ibid.*, **58**, 1179 (1936).

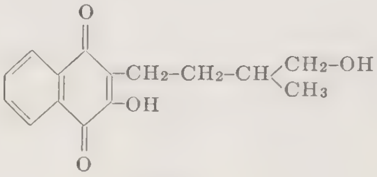
[47] Fieser: *J. Am. Chem. Soc.*, **49**, 857 (1927).

[48] Lee: *Proc Chem. Soc. (London)*, **17**, 4 (1901): *J. Chem. Soc.*, **79**, 284 (1901); Peckolt: *Ber. deut. pharm. Ges.*, **22**, 24 (1912); Oesterle: *Schweiz. Wochschr. Chem. Pharm.*, **50**, 529 (1912); *Arch. Pharm.*, **251**, 301 (1912); *Bloemendal: Pharm. Weekblad.*, **43**, 678 (1906).

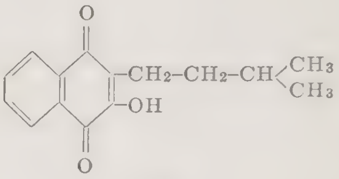
[49] Rennie: *J. Chem. Soc.*, **67**, 787 (1895); Hooker: *J. Chem. Soc.*, **69**, 1381 (1896).



(I)

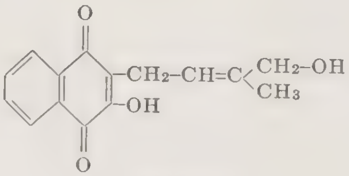


(II)



(III)

Both lomatiol and lapachol thus contain the same arrangement of carbon atoms in the side-chain. Taking its other properties into consideration, therefore, lomatiol has the constitution:



Lomatiol

The pigment is isolated by extracting the seeds with dilute acetic acid and allowing the solution to crystallize. Lomatiol exhibits the activity of vitamin K (see below).

Alkannin is contained in the root of *Alcanna tinctoria* (*Anchusa tinctoria*) a plant of the *Boraginaceae* family which flourishes in Cyprus, Peloponnesus, Hungary, Italy and Spain. It is known also under the names pseudo or false alcanna,[51] ox-tongue root, stone-weed and orcanella. Alkannin[52] forms red-brown needles with a coppery luster (m.p. 148°) and has the composition corresponding to $C_{16}H_{16}O_5$, although it seems to be bound in the plant to angelic acid.[53] The constitution was elucidated by Brockmann who was able to show that alkannin and shikonin (q.v.) are optical enantiomorphs. Alkannin contains three active hydrogen atoms and thus yields a triacetyl derivative (yellow needles, m.p. 132°), but only a dibenzoyl compound (yellow needles, m.p. 174-

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[50] Hooker: *J. Am. Chem. Soc.*, **58**, 1181 (1936); Hooker, Steyermark: *Ibid.*, 58, 1198, 1207 (1936).

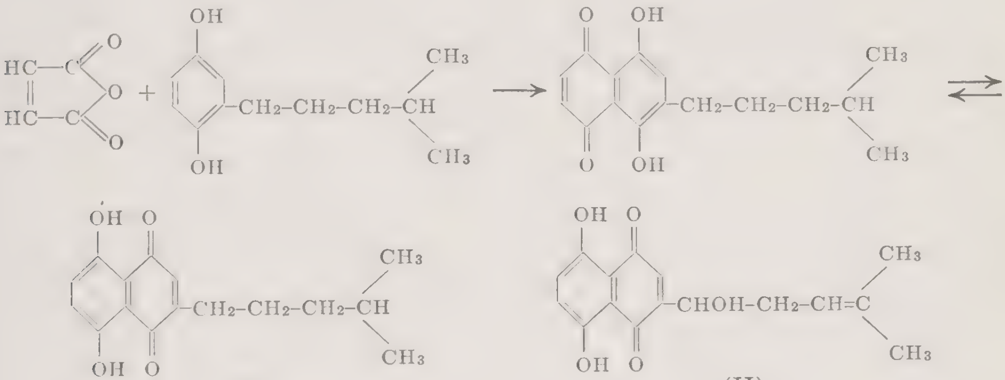
[51] It is distinguished thus from the true alkanna root from *Lawsonia alba*.

[52] Brockmann, Ross: *Naturwiss.*, **23**, 246 (1935); *Ann.*, **521**, 1 (1936); see also, Raudnitz, Stein: *Ber.*, **68**, 1479 (1935): older work which has been largely revised: Carnelutti, Nasini: *Ber.*, **13**, 1514 (1880); Liebermann, Römer: *Ber.*, **20**, 2428 (1887); Dieterle, Salomon, Nosseck: *Ber.*, **64**, 2086 (1931); Raudnitz, Fiedler, Redlich: *Ber.*, **64**, 1835 (1931); Raudnitz: *Ber.*, **65**, 159 (1932); Betrabet, Chakravarti: *J. Indian Inst. Sci. A*, **16**, 41 (1933).

[53] Brand, Lohmann: *Ber.*, **68**, 1487 (1935).

175°) has been obtained. Its absorption spectrum suggested a derivative of naphthazarin, and the presence of an isopropylidene group was also established.

Alkannin is optically active $[\alpha]_{\text{Cd}} = -167°$ (benzene) the asymmetric carbon atom carrying a hydroxyl group in the side chain. The carbon skeleton was indicated by the formation of naphthalene and α-methylanthracene on distillation with zinc dust, and of 1-methylquinizarin on sublimation. Still more significant was the isolation by sublimation of alkannin in high vacuum of alkannan (I), $C_{16}H_{18}O_4$, 3-isohexylnaphthazarin, the constitution of which was confirmed both by oxidative degradation and the following synthesis[54]:



(I) (II)

Alkannin therefore possesses the structure (II) and the formation of the anthracene derivative on distillation with zinc dust is due to a ring closure.

Alkannin dyes material mordanted with aluminum a violet shade and was formerly used to dye cotton and silk. At present it finds some application in the cosmetic industry as a coloring matter and it is recommended also as an indicator[55] and as a reagent for magnesium.[56]

Tokyo Violet. This violet coloring matter, first investigated by Kuhara[57] and later by Majima and Kuroda,[58] occurs in the root of *Lithospermium Erythrorhizone* which is known in northeastern Japan under the name Shikone. It has the empirical formula $C_{18}H_{18}O_6$ (m.p. 85-86°), and its isolation presents considerable difficulty, as it crystallizes from an extract in petroleum ether only after standing for about a year. On extracting the petroleum solution however, and then adding dilute sulfuric acid, a product, shikonin, $C_{16}H_{16}O_5$ (m.p. 147°) crystallizes in violet-brown needles. Shikonin lacks an acetyl group present in the parent shikone and forms a disodium salt and a triacetyl derivative, yellow needles (m.p. 168°).

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[54] Kuroda, Wada: *Proc. Imp. Akad. (Tokyo)*, 12, 239 (1936); *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, 34, 1740 (1938); Brockmann, Müller: *Ann.*, 540, 51 (1939).

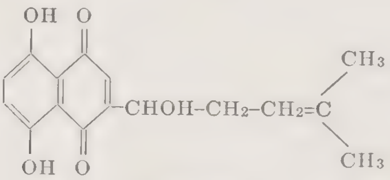
[55] Boettger: *J. prakt. chem.*, 107, 46 (1869); Enz: *Jahresb. v. Liebig u. Kopp*, 1870, 935 (1879).

[56] Eisenlohr: *Ber.*, 53, 1476 (1920).

[57] Kuhara: *Chem. News*, 38, 238 (1878); *Ber.*, 11, 2146 (1878).

[58] Majima, Kuroda: *Acta phytochimica (Japan)*, 1, 43 (1922); Kuroda, Wada: *Sci. Papers Inst. hys. Chem. Research (Tokyo)*, 34, 1740 (1938).

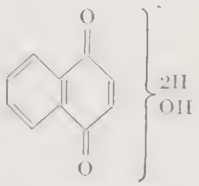
Reductive acetylation results in the introduction of five acetyl groups, and dry distillation yields 1-methyl-5-8-dihydroxyanthraquinone [59] (shikizarin); and naphthalene, α- and β-methylanthracene occur among the products obtained by distilling with zinc dust. These facts all found explanation when Brockmann [60] recognized that shikonin is the optical antipode of alkannin and possesses the same structural formula:



Shikonin

A racemate of alkannin and shikonin also occurs in nature and is known as shikalkin. The pigment is isolated by extracting the roots and shoots with petroleum, and evaporating and purifying the residue. Twenty kg of root yield 1260 g of syrup, and 700 g of syrup give 435 g of shikonin.

Pigment of Drosera Binata. A pigment which was regarded by Fünfstück and Braun [61] as having a structure closely related to that of juglone is found in the roots and petioles of Drosera binata. According to Dieterle [62] it crystallizes in golden-yellow rhombic needles (m.p. 106-108°) and its alcoholic solution assumes an amethyst color in the presence of sodium hydroxide, and becomes red-brown on addition of ferric chloride. It is stated to have the formula $C_{10}H_8O_3$, although verification of this would be desirable. The compound contains one hydroxyl group (Zerewitinoff determination) and it forms a p-nitrophenylhydrazone (m.p. 174-175°); oxidation yields a hydroxyphthalic acid, so that the following formula may be provisionally assigned:



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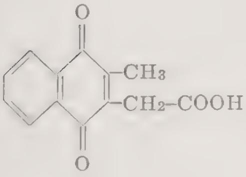
[59] Hayashi: *J. Chem. Soc.*, 1927, 2516; F. Mayer, Stark: *Ber.*, 64, 2003 (1931).

[60] Brockmann: *Naturwiss.*, 1935, 246; *Ann.*, 521, 1 (1935).

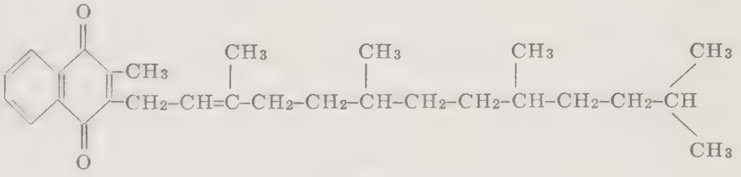
[61] Fünfstück, Braun: *Ber. deut. bot. Ges.*, 34, 160 (1916); Eichhorn *(Bot. Inst. Tech. Hochsch. Stuttgart)* was the first to describe the compound.

[62] Dieterle: *Arch. Pharm.*, 260, 45 (1922); *Apoth. Z.*, 42, 396 (1939); Karrer: *Helv. Chim. Acta*, 22, 1146 (1939); Thayer, Binkley MacCorquodale, Doisy, Emmett, Brown, Bird: *J. Am. Chem. Soc.*, 61, 2563 (1939); Tishler, Sampson: *Ibid.*, 61, 2563 (1939); Fieser, Campbell, Fry, Gates, Jr.: *Ibid.*, 61, 2559 (1939); Almquist, Klose: *Ibid.*, 61, 2557 (1939).

Vitamin K (K_1),[63] the antihemorrhagic factor of the chick, has recently been shown to belong to the group of naphthoquinones. The American workers suggested a quinonoid structure from the yellow color of the vitamin and the formation of a diacetate on reductive acetylation. On catalytic hydrogenation vitamin K_1 takes up four molecules of hydrogen, three of which are employed in reducing the (naphtho-) quinone ring while the fourth reduces an unsaturated side-chain. The nature of the side-chain and the location of the ethylenic linkage are indicated by ozonolysis, whereby a ketone is obtained which is probably identical with 2,6,10-trimethylpentadecan-14-one. The postulated naphthoquinone nucleus receives support from more drastic degradation with chromic acid when phthalic acid and a second product, 2-methyl-1,4-naphthoquinone-3-acetic acid (I), are obtained. For vitamin K_1 the same nucleus is suggested and its constitution is given as 2-methyl-3-phytyl-1,4-naphthoquinone (II).[63]



(I)



(II)

Vitamin K_2,[64] from putrified sardine meal, is assigned on similar evidence a structure as a 2-methyl-3-difarnesyl-1,4-naphthoquinone (1).

These findings, and the fact that phthiocol exhibits weak vitamin K activity at high dosage levels[65] led to the discovery that the antihemorrhagic function is not a specific property of any one chemical structure but is shared by a number of synthetic 1,4-naphthoquinones.[66] Of particular interest is the observation that 2-methyl-1,4-naphthoquinone is about three times as potent as vitamin K, on a weight basis.[66a] According to Kuhn and his collaborators a certain degree of vitamin K

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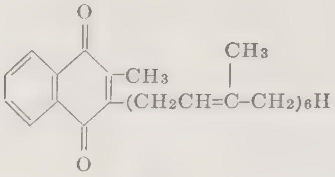
[63] Literature: McKee, Binkley, MacCorquodale, Thayer, Doisy: *J. Am. Chem. Soc.*, **61**, 1295 (1939); McKee, Binkley, Doisy, MacCorquodale, Thayer, Cheney: *Ibid.*, **61**, 1613 (1939); Almquist, Klose: *Ibid.*, **61**, 1611 (1939); Ansbacher, Fernholz: *Ibid.*, **61**, 1924 (1939); L. Fieser, Bowen, Campbell, M. Fieser, Fry, Jones, Riegel, Schweitzer, Smith: *Ibid.*, **61**, 1925 (1939); MacCorquodale, Binkley, Thayer, Doisy: *Ibid.*, **61**, 1928 (1939); Thayer, Cheney, Binkley, Doisy, MacCorquodale: *Ibid.*, **61**, 1932 (1939); L. Fieser, Fry, Campbell: *Ibid.*, **61**, 2206 (1939). Synthesis: L. Fieser, *Ibid.*, **61**, 2559, 3467 (1939); Binkley, Cheney, Holcomb, McKee, Thayer, Doisy, MacCorquodale: *Ibid.*, **61**, 2558 (1939).

[64] McKee, Binkley, MacCorquodale, Thayer, Doisy: *J. Am. Chem. Soc.*, **61**, 1295 (1939); Binkley, McKee, Thayer, Doisy, *J. Biol. Chem.*, **133**, 721 (1940).

[65] Almquist, Klose: *Ibid.*, **61**, 1611 (1939).

[66] Kuhn, Wallenfels, Weygand, Moll, Hepding: *Naturwiss.*, **27**, 518 (1939).

[66a] Ansbacher, Fernholz, *J. Am. Chem. Soc.*, **61**, 1924 (1939).



(I)

activity is shown by echinochrome (q.v.) and a number of purely synthetic quinones, including derivatives of benzoquinone, but it is now recognized that the apparent activity of compounds of these types is too slight to be of any great biological significance.

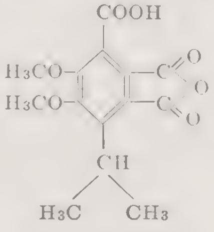
The more recent literature contains a considerable bulk of work which is not reviewed at length here, as it is concerned rather with the synthesis of substitutes for vitamin K.[66b] Briefly, the results show that a methyl group in the 2-position of the naphthoquinone nucleus is particularly favorable for the appearance of vitamin activity; 2-methylnaphthoquinone is used clinically, though medical opinion does not appear to be wholly satisfied that its use is not accompanied by harmful secondary effects; in this connection it is interesting to note that it has been suggested that the superior activity of 2-methylnaphthoquinone is only apparent; methylnaphthoquinone may function as a precursor of the phytyl compound since, when due account of its lower molecular weight is taken, the superiority disappears.

Gossypol,[67] the pigment of the cotton seed, exists in three crystalline modifications (m.p. 184°, 199°, and 214°). It does not occur as a glycoside and its formula is now accepted as $C_{30}H_{30}O_8$. Gossypol forms a molecular compound with acetic acid, gossypol acetate, which facilitates its purification. It dissolves in alkali to a yellow solution, assuming after a short time a violet color which then becomes weaker and finally fades completely. The same behavior, which indeed is due to oxidation, is observed with hydrogen peroxide. Of the very considerable volume of research which has been carried out to determine the constitution of gossypol, only that of Adams need be briefly discussed here as it has resulted in a formula being assigned and naturally embraces the results of earlier workers which have served as a basis.

[66b] L. Fieser, Tishler, Wendler: *J. Biol. Chem.*, **137**, 659 (1941).

[67] Marchlewski: *J. prakt. Chem. (2)*, **60**, 85 (1899); Carruth: *J. Am. Chem. Soc.*, **40**, 647 (1918); Clark: *J. Biol. Chem.*, **75**, 725 (1927); **76**, 229 (1928); **77**, 81 (1928); **78**, 159 (1928); *J. Am. Chem. Soc.*, **51**, 1475, 1479 (1929); *Oil & Fat Industries*, **6**, No. 7, 15 (1929); Karrer, Tobler: *Helv. Chim. Acta*, **15**, 1204 (1932); Schmid, Margulies: *Monatsh.*, **65**, 391 (1935); Samyschliajewa: *Oil Fat Ind. (U.S.S.R.)*, **13**, 2 (1937); *Chem. Zentr.*, **1937**, II, 1828; Miller, Butterbaugh, Adams: *J. Am. Chem. Soc.*, **59**, 1729 (1937); Morris, Adams: *Ibid.*, **59**, 1731 (1937); Miller, Adams: *Ibid.*, **59**, 1736 (1937); Adams *et al.*: *Ibid.*, **60**, 2158, 2160, 2163, 2166, 2170, 2174, 2184, 2188, 2193, 2967, 2970, 2972 (1938); Podolskaja: *Biochem. Z.*, **284**, 401 (1936); *Oil Fat Ind. (U.S.S.R.)*, **13**, 8 (1937); *Chem. Zentr.*, **1938**, *I*, 4665; Campbell, Morris, Adams: *J. Am. Chem. Soc.*, **59**, 1723 (1937). Absorption spectrum: Grinbaumowna, Marchlewski: *Biochem. Z.*, **286**, 295 (1936); *Bull. intern. acad. polon. sci. Classe sci. math. nat. A*, **1936**, 367; *Chem. Zentr.*, **1937**, *I*, 835; Samyschlajewa, Kriwitsch: *J. Gen. Chem. (U.S.R.R.)*, **7** (69), 1969 (1937); *Chem. Zentr.*, **1938**, I, 4340; Adams, Kirkpatrick: *J. Am. Chem. Soc.*, **60**, 2180 (1938). Determination of gossypol: Smith, Halverson, *Ind. Eng. Chem. (Anal. Ed.)*, **11**, 475 (1939).

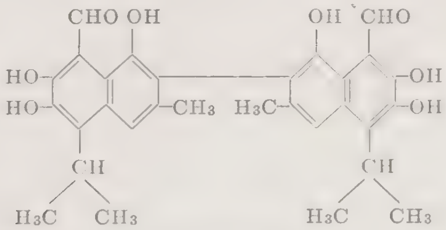
Gossypol reacts with aromatic amines with the formation of condensation products of the type of Schiff's bases. These condensations are due to two aldehyde radicals which are theoretically oxidizable to the hypothetical "gossylic acid." A similar oxidation has been realized in the conversion of gossypol hexamethyl ether—the remaining six oxygen atoms in gossypol are attacked as hydroxyl groups, as is evidenced by the introduction of six methyl groups—into, among other products, gossic acid, $C_{14}H_{14}O_7$:



Gossic Acid

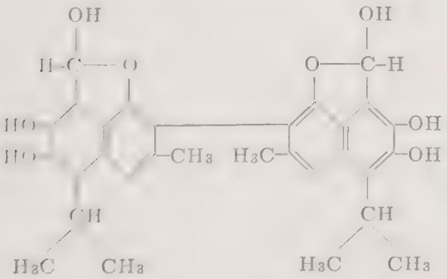
Gossypol tetramethyl diethyl and tetraethyl dimethyl ethers have also been described. These resemble the hexamethyl ether and can be oxidized and reduced to analogous compounds.[67a]

More closely investigated have been the derivatives of apogossypol, $C_{28}H_{30}O_6$, which is obtained by the action of hot sodium hydroxide on gossypol and which has led to the formulation of the latter as 1,6,7,1′,6′,7′-hydroxy-3,3′-dimethyl-5,5′-diisopropyl-2,2′-dinaphthyl-8,8′-dialdehyde:

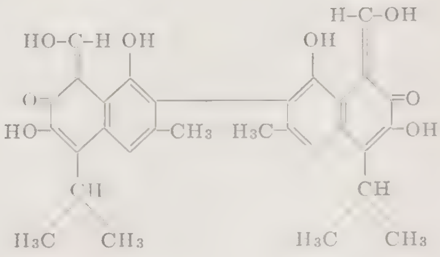


Gossypol

which may be written in the tautomeric forms:



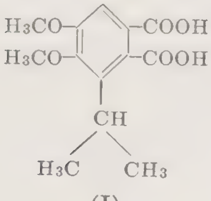
and



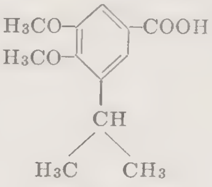
[67a] Adams, Dial, *J. Am. Chem. Soc.*, 61, 2077 (1939).

Apogossypol contains the same carbon skeleton as gossypol but lacks the two aldehyde groups.

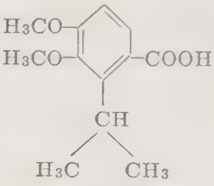
The orientation of the substituents has recently been partially confirmed synthetically. If the postulated structures are correct, apogossypolic acid [68] must be (I) and the monocarboxylic acid formed on heating it with hydrobromic acid must be (II) or (III):



(I)

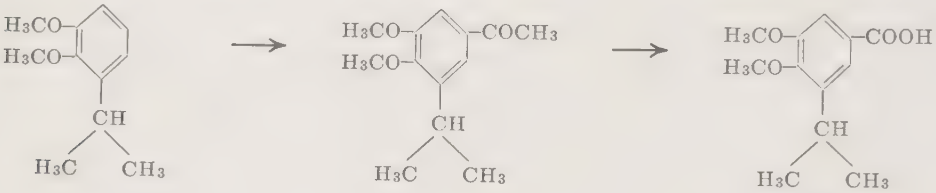


(II)

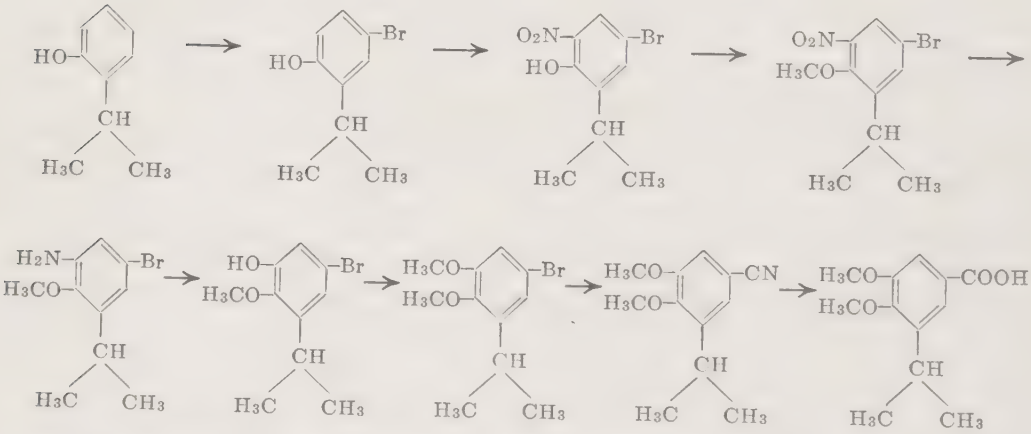


(III)

The 6-carboxylic acid (III) was found to be not identical with the product of natural origin, which however agreed in properties with (II) synthesized in the following mutually confirmatory ways:



(II)



The 1,2-dihydroxy-3-isopropyl arrangement in gossypol is thus established, and the fusion of the rings in the 4,5-positions, which is also proved by the synthesis of this acid, is further confirmed by the synthesis of the dimethyl ether of apogossypolic acid itself:

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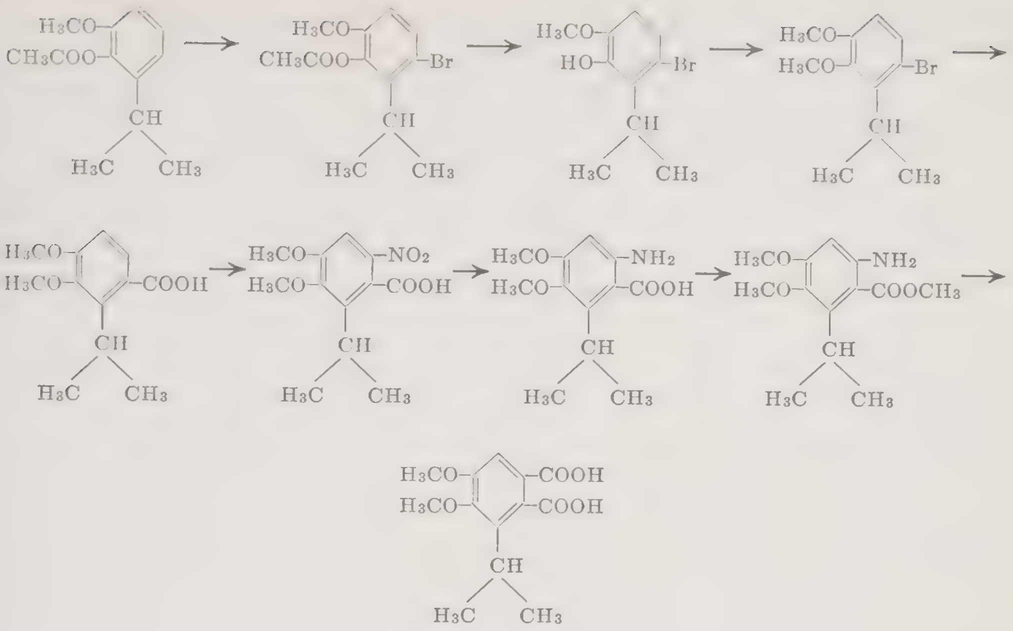
[68] Adams, Hunt, Baker: *J. Am. Chem. Soc.*, 61, 1134 (1939); Adams, Baker: *Ibid.*, 61, 1138 (1939).



Gossypol is obtained[69] by extracting cottonseed cake with petroleum ether and precipitating the extract with acetic acid. Gossypol[70] possesses poisonous properties.

Anthracene Compounds

Pigments of this class are found in nature in the roots of a number of *Rubiaceae*, particularly in *Rubia tinctorum*, the common madder of Europe and Asia, in rhubarb (*Rheum*) and aloes, in *Rhamnus* species, in lichens (*e.g.*, in *Parmelia parientina*) and in fungi. They very frequently occur in the plant in the form of glycosides.[71]

The isolation of the chief tinctorial principle of *Rubia tinctorum*, the madder dyestuff alizarin, was a process of importance because of the brilliance and fastness of the dye, and the plant was formerly very widely cultivated.[72] The coloring matter of rhubarb and aloes are of significance only in pharmaceutical connections.[73] Of unusual interest is the occurrence of an unknown polyhydroxyanthraquinone derivative in the mineral kingdom.[74] In the animal kingdom three so-called insect pigments to which a separate section is devoted below, *i.e.*, cochineal, kermes and litmus, were of considerable technical importance before the introduction of synthetic dyestuffs.

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[69] Recent procedures: Podolskaja: *Fettchem. Umschau,* **42,** 96 (1935); *Chem. Zentr.,* **1935,** II, 2149; Campbell, Morris, Adams: *J. Am. Chem. Soc.,* **59,** 1723 (1937); Koshewnikow, Giltburg: *Oil Fat Industries (U.S.S.R.),* **12,** 545 (1936); *Chem. Zentr.,* **1937,** II, 690; Giltburg: *Oil Fat Industries (U.S.S.R.),* **12,** 546 (1936); *Chem. Zentr.,* **1937,** *II,* 690.

[70] Gallup: *J. Biol. Chem.,* **93,** 381 (1931); Clark: *Oil & Fat Industries,* **5,** 237, 273 (1928).

[71] Wehmer, Thies: "Systematische Verbreitung und Vorkommen der Anthracenglucoside," in Klein, "Handbuch der Pflanzenanalyse," **III,** 2, p. 1033.

[72] Ullmann, "Encyclopaedie der technischen Chemie," 2nd Ed., **V,** p. 135.

[73] *Tiba,* **9,** 711 (1931).

[74] Treibs, Steinmetz: *Ann.,* **506,** 171 (1933).

The suggestion that anthracene compounds are formed in the plant from hydroxybenzene carboxylic acids, as in the synthesis of anthragallol from benzoic and gallic acids,[75] by enzyme reaction alone, warrants some attention. More in keeping with modern conceptions, perhaps, is the schematic synthesis from units such as benzoquinone and isoprene, with subsequent dehydrogenation.[76] A number of empirical rules have been formulated by Mitter and Biswas [77] from which the orientation of anthraquinone derivatives occurring in nature may be deduced. They consider four basic types occurring in the vegetable kingdom, but it must be mentioned that a number of pigments are known which cannot be included in their problematic systems.

Alizarin is found in the form of a glycoside, rubierythric or ruberythric acid, in the madder-root,[78] and in the root of *Olenlandia umbellata*. Rubierythric acid has the composition corresponding to $C_{25}H_{28}O_{14}$ (Richter [79]) (formerly given as $C_{26}H_{28}O_{14}$) and forms yellow needles (m.p. 257°). Graebe, Liebermann and Bergami [80] held the view that the acid is a diglycoside; and a variety of glycosides, such as an alizarin monoglucoside of Takahashi,[81] alizarin-2-β-gentiobioside, and alizarin cellobioside,[82] none of which is identical with rubierythric acid,[83] have been prepared for comparative purposes. Similarly the properties of the octa-acetate of alizarin maltoside [84] do not agree with those of the natural derivative. That no diglycoside of alizarin has yet been obtained which dissolves in alkali with formation of a salt, as does rubierythric acid,[85] is an indication that the latter is not a diglycoside but a bioside.

More recent work by Jones and Robertson [86] has shown that rubierythric acid must be a β-glycoside, as it is hydrolyzed by emulsin; moreover, after hydrolysis, positive orcinol and phloroglucinol reactions for a pentose were obtained. Finally, Richter [79] was able to show that the sugar constituent is primeverose and that it is attached to the 2-hydroxyl group, as on methylating rubierythric acid and then subjecting it to hydrolysis by acid, alizarin-1-methyl ether was obtained. Rubierythric acid is thus:

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[75] Seuberlich: *Ber.*, **10**, 38 (1878).

[76] Diels, Alder: *Ann.*, **460**, 98 (1928); *Ber.*, **62**, 2337 (1929); D.R.P. 494,433, 496,393 (I. G.), Friedländer, **16**, 1201; Carré, Libermann: *Compt. rend.*, 199, 791 (1934); *Bull. soc. chim. (5)*, **2**, 291 (1935). On the technical importance of synthetic dyestuffs of this series, cf. Fritz Mayer, "Chemie der organischen Farbstoffe," Vol. **1**, Berlin, 1934.

[77] Mitter, Biswas: *J. Indian Chem. Soc.*, **5**, 769 (1928); cf. also, "Review of synthesis of naturally occurring anthraquinone derivatives"; Mitter: *J. Indian Chem. Soc.*, P. C. Ray Commemorial Volume, 285 (1933); cf. also Eder, Siegfried: *Pharm. Acta Helv.*, **14**, 34 (1939).

[78] The term "madder" denotes the ground madder-root containing about 4 per cent of coloring matter. Exhaustive bibliography: Schultz: "Farbstofftabellen" 7 ed., Vol. **I**, p. 638, No. 1379.

[79] Richter: *J. Chem. Soc.*, **1936**, 1701.

[80] Graebe, Liebermann: *Ann. (Suppl.)*, **7**, 296 (1870); Liebermann, Bergami: *Ber.*, **20**, 2241 (1887); Bergami: *Ber.*, 20, 2247 (1887).

[81] Takahashi: *J. Pharm. Soc. Japan*, No. **525**, 4 (1925); cf. Glaser, Kahler: *Ber.*, **60**, 1349 (1927).

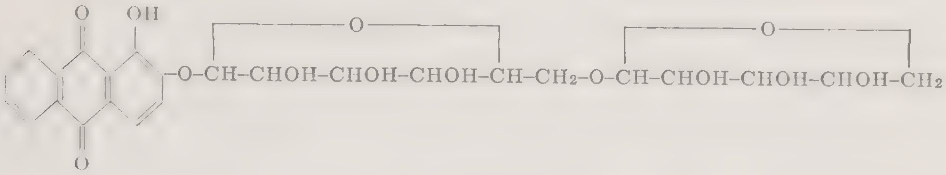
[82] Zemplén, Müller: *Ber.*, **62**, 2107 (1929).

[83] Jones, Robertson: *J. Chem. Soc.*, **1933**, 1167.

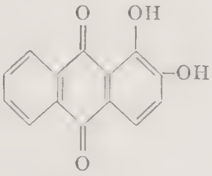
[84] Robertson: *J. Chem. Soc.*, **1930**, 1136.

[85] Müller: *Ber.*, **62**, 2793 (1929).

[86] Jones, Robertson: *J. Chem. Soc.*, **1933**, 1167; Zemplén, Bognar: *Ber.*, **72**, 913 (1939).



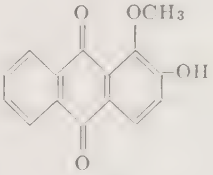
It was synthesized after many unsuccessful attempts by the interaction of acetobromprimeverose and alizarin. This structure for rubierythric acid has now been confirmed[86a] by a synthesis along orthodox lines from acetobromoprimeverose and alizarin. The hexaacetyl compound so obtained was acetylated to β-2-heptaacetylalizarin primeveroside and loss of the acetyl groups gave rubierythric acid.



Alizarin

Alizarin, $C_{14}H_8O_4$, 1:2-dihydroxy-anthraquinone, forms red needles (m.p. 289-290°) and dyes wool mordanted with chromium a red-brown, and turkey-red mordanted cotton a fiery red. Rubierythric acid is prepared by extracting madder root with boiling water, precipitating with lead acetate and further purifying the liberated acid.

Alizarin-α-methyl ether (I), $C_{15}H_{10}O_4$ (m.p. 178-179°), is found in *Morinda longiflora*[87] and *citrifolia*[88] and in *Olenlandia umbellata,*[89] in the latter probably as rubierythric acid methyl ether.[90] It is extracted by sulfurous acid.



(I)

[86a] Zemplén: *Math. Naturw. Anz. Akad. Wiss.*, 58, 380 (1939).

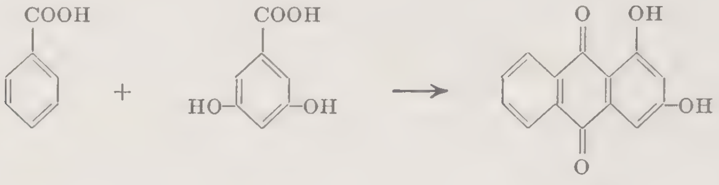
[87] Barrowcliff, Tutin: *J. Chem. Soc.*, 91, 1907 (1907).

[88] Simonsen: *Ibid.*, 117, 561 (1920).

[89] A. G Perkin. Hummel: *Ibid.*, 63, 1174 (1893); 67, 817 (1895).

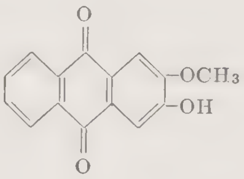
[90] Richter: *Ibid.*, 1936, 1701.

Purpuroxanthin[91] (xanthopurpurin) (II), $C_{14}H_8O_4$ (yellow needles, m.p. 270°), accompanies purpurin in madder. It has been synthesized from benzoic and sym.-dihydroxybenzoic acids[92]:

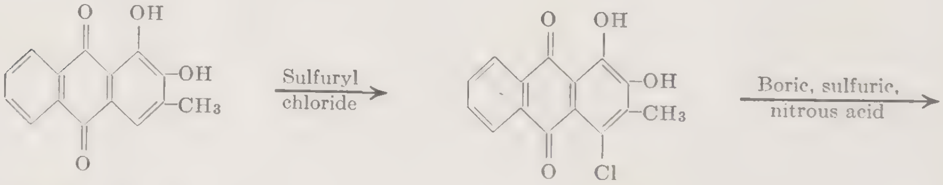


(II)

Hystazarin monomethyl ether[93] (III), $C_{15}H_{10}O_4$ (orange-yellow needles, m.p. 239°), is found in *Olenlandia umbellata* and may be prepared from hystazarin dimethyl ether.



Munjistin (purpuroxanthin carboxylic acid),[94] $C_{15}H_8O_6$ (golden-yellow leaflets, m.p. 231°). The chief sources of this pigment are the roots of *Rubia munjista tinctorum* and *siccimensis,* where it occurs as a glycoside; it is accompanied by an isomeride,[95] $C_{15}H_8O_6$, which crystallizes in red needles (m.p. 202°). It also occurs in the mother-liquors after separating crude purpurin from madder.[96] Munjistin has been synthesized[97] by the following reactions, although the yields are so unsatisfactory that too little of the final product was obtained to carry out an analysis:



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[91] Literature: Houben: "Anthracen and Anthrachinone," Leipzig, 1929.

[92] Noah: *Ber.*, **19,** 332 (1886).

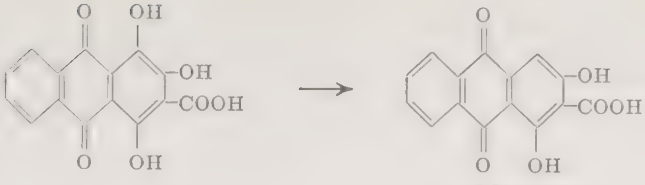
[93] A. G. Perkin, Hummel: *J. Chem. Soc.*, **67,** 817 (1895); A. G. Perkin: *Ibid.*, **91,** 2066 (1907).

[94] Stenhouse: *Ann.*, **130,** 325 (1884); Schunck, Römer: *Ber.*, **10,** 172, 790 (1877); *J. Chem. Soc.*, **31,** 666 (1877); **33,** 422 (1878); Plath: *Ber.*, **10,** 614 (1877); Perkin, Hummel: *J. Chem. Soc.*, **63,** 1157 (1893).

[95] Perkin, Hummel: *Ibid.*, **63,** 1157 (1893).

[96] Schunck, Römer: *Ber.*, **10,** 172, 790 (1877).

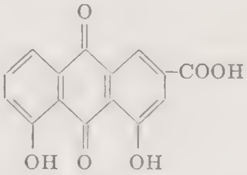
[97] Mitter, Sen: *J. Indian Chem. Soc.*, **5,** 631 (1928); Mitter, Biswas: *Ibid.*, **7,** 839 (1930); *Nature,* **126,** 761 (1930); **127,** 166 (1931); *Ber.*, **65,** 622 (1932).



Munjistin

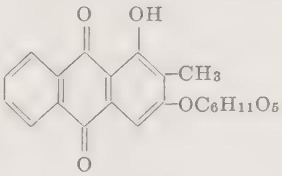
Munjistin dyes material mordanted with aluminum an orange shade.

Rhein,[98] $C_{15}H_8O_6$ (yellow needles, m.p. 312°), is found in Chinese rhubarb from which a related compound, rheinolic acid, has also been isolated. Rheinolic acid,[99] $C_{17}H_{10}O_6$, forms deep-red needles, (m.p. 295-297°; acetyl derivative, m.p. 236°). Rhein is best obtained from Chinese rhubarb [100] or from barbaloin by oxidation. Its constitution [101] is deduced from that of chrysophanic acid (q.v.) which may be converted into rhein.



Rhein

Rubiadin,[102] $C_{21}H_{20}O_9$ (yellow needles, m.p. 270°), has been isolated from madder in the form of a glucoside but probably occurs in the plant in a more complex form. The glucoside residue is attached to the 3-hydroxyl group as in (I) and undergoes hydrolysis to glucose and rubiadin on heating with dilute mineral acid. Rubiadin occurs also as a primoveroside,[103], $C_{26}H_{28}O_3$ (yellow leaflets, m.p. 248-250°), in *Lalium verum.*



(I)

The constitution [104] of the parent rubiadin, $C_{15}H_{10}O_4$, which forms yellow needles (m.p. 290°), has been confirmed by several syntheses[105] such as from benzoic acid and 1-methyl-2,6-dihydroxy-4-carboxylic acid:

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[98] Literature: Beilstein: Vol. **X**, p. 1033.

[99] Tutin, Clewer: *J. Chem. Soc.*, **99**, 954 (1911).

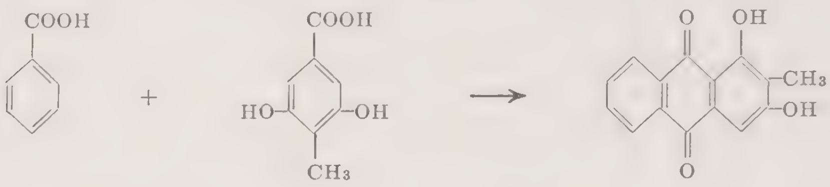
[100] Tschirch, Heuberger: *Arch. Pharm.*, **240**, 596 (1902).

[101] Oesterle, Tisza: *Schweiz. Wochschr Chem. Pharm.*, **46**, 701 (1908); Robinson, Simonsen: *J. Chem. Soc.*, **95**, 1085 (1909); Oesterle, Riat: *Arch. Pharm.*, **247**, 413 (1909); Oesterle: *Ibid.*, **250**, 305 (1912); Oesterle: *Schweiz. Wochschr. Chem. Pharm.*, **49**, 661 (1911); Eder, Widmer: *Helv. Chim. Acta*, **5**, 3 (1922).

[102] Schunck, Marchlewski: *J. Chem. Soc.*, **63**, 969 (1893); **65**, 182 (1894); Jones, Robertson: *Ibid.*, **1930**, 1699.

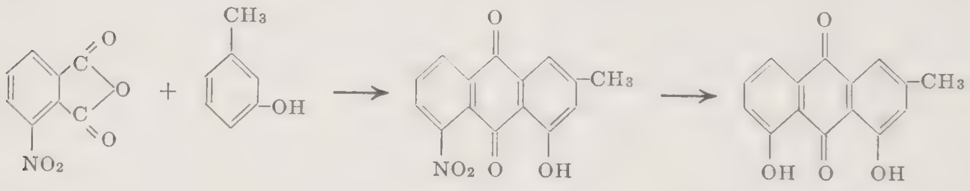
[103] Hill, Richter: *Ibid.*, **1936**, 1714.

[104] Stouder, Adams: *J. Am. Chem. Soc.*, **49**, 2043 (1927): Mitter, Sen, Paul: *Quart. J. Indian Chem. Soc.*, **4**, 535 (1927); Kuroda: *J. Pharm. Soc. Japan*, **55**, 110 (1935).



Rubiadin-1-methyl ether (yellow plates, m.p. 291°), which occurs naturally in *Morinda longiflora*[106] and *Morinda citrifolia*,[107] has been obtained synthetically.[108] Rubiadin is most conveniently obtained by precipitating an aqueous extract of madder root with lead acetate and hydrolyzing the glucoside so isolated.

Chrysophanic acid (chrysophanol)[109] (I) occurs in *Rheum officinale*, *Rheum rhaponticum*, *Rumex obtusifolius*, *Rumex nepalensis*, *Rumex eclonianus*, in the lichen *Parmelia parietina*, in senna leaves, in *Cassia bijuga*, *Rhamnus frangula*,[110] etc. It is present in Chinese rhubarb as the glycoside chrysophanein, $C_{21}H_{20}O_9$ (yellow needles, m.p. 242-249°) which occurs also together with rheochrysin, emodin diglycoside and rhein glycoside as rheopurgarin. Chrysophanic acid, $C_{15}H_{10}O_4$ (I), crystallizes in golden-yellow leaflets (m.p. 196°) and dissolves in alkali to a cherry-red solution. It is constituted[111] as 4,5-dihydroxy-2-methylanthraquinone and has been synthesized from 3-nitrophthalic anhydride and *m*-cresol[112]:



(I)

This synthesis, it will be noticed, is not unambiguous, as the first condensation may yield two isomeric products:

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[105] Mitter: *Nature*, **120**, 729 (1927); Mitter, Gupta: *J. Indian Chem. Soc.*, **5**, 25 (1928); Mitter, Pal: *Ibid.*, **7**, 259 (1930); Jones, Robertson: *J. Chem. Soc.*, **1930**, 1699.

[106] Barrowcliff, Tutin: *J. Chem. Soc.*, **91**, 1907 (1907).

[107] Barrowcliff, Tutin: *Ibid.*, **91**, 1907 (1907).

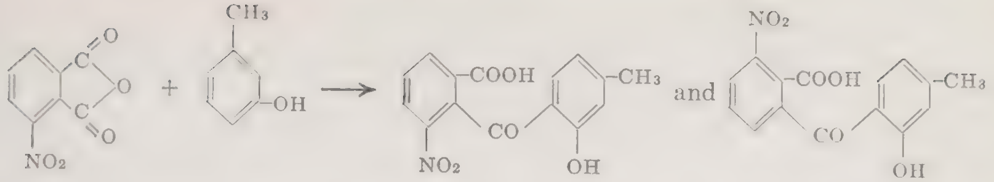
[108] Jones, Robertson: *Ibid.*, **1930**, 1699.

[109] Older literature: Beilstein: Vol. **VIII**, 470; for constituents of Indian rhubarb, cf. Mohiuddin, Katti: *J. Indian Inst. Sci. A*, **16**, 1 (1933).

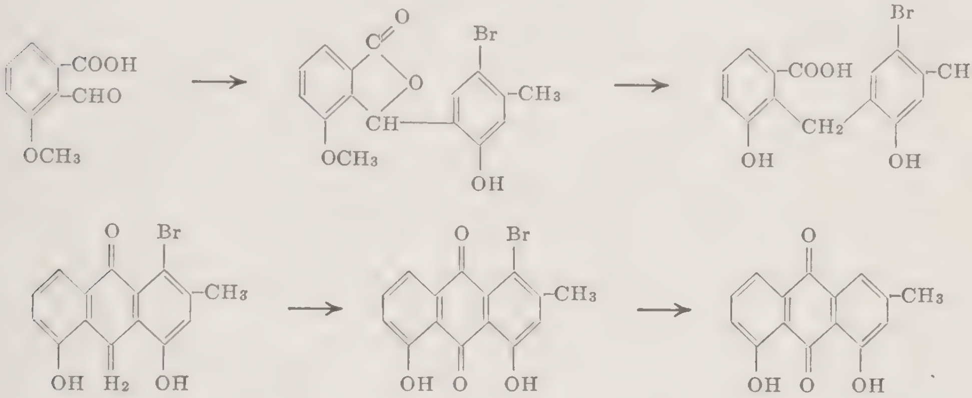
[110] Bridel, Charaux: *Bull. soc. chim. biol.*, **8**, 655 (1926).

[111] Léger: *Compt. rend.*, **153**, 114 (1911); **154**, 281 (1912); *J. pharm. chim. (7)*, **5**, 281 (1912); Oesterle: *Arch. Pharm.*, **250**, 301 (1912).

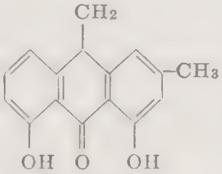
[112] Eder, Widmer: *Helv. Chim. Acta*, **5**, 3 (1922); **6**, 419 (1923); D. R. P. 397,316 (Eder) Frdl. **14**, 1446.



It has, however, been confirmed in this respect by a synthesis starting from 3-methoxyphthalic acid aldehyde and 2-brom-5-cresol[113]:



Chrysophanic acid dyes wool mordanted with chromium a red shade. Chrysophanein[114] is best isolated by starting from rheopurgarin. The so-called chrysophanol of Araroba or Goa powder which is known by the name chrysarobin[115] and is often wrongly termed chrysophanic acid, is a mixture of 4,5-dihydroxy-2-methyl-10-anthrone (I) (pale yellow needles, m.p. 205-210°) with reduction products of emodin and emodin methyl ether. Chrysophanic acid may yield two anthrones, but the constitution (I) assigned to the compound of chrysarobin has been confirmed by comparison with a synthetic product:



(I)

Purpurin,[116] $C_{14}H_8O_5$ (orange needles, m.p. 256°), occurs, probably as a glycoside, in madder. A purpurin lake with alumina is bright scarlet-red in color. Purpurin has been obtained by oxidizing alizarin.

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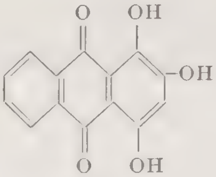
[113] Naylor, Jr., Gardner: *J. Am. Chem. Soc.*, **53**, 4109, 4114 (1931).

[114] Eder, Widmer: *Helv. Chim. Acta*, **5**, 17 (1922).

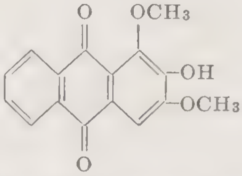
[115] Rochleder: *Ber.*, **2**, 373 (1869); Hesse: *Ann.*, **309**, 32 (1899); Tschirch, Christofoletti: *Arch. Pharm.*, **243**, 443 (1905); O. Fischer, Gross: *J. prakt. Chem. (2)*, **84**, 369 (1911); Naylor, Jr., Gardner: *J. Am. Chem. Soc.*, **53**, 4114 (1931).

[116] Literature: Schultz, "Farbstofftabellen," 7th ed., Vol. **I**, p. 510, No. 1157.



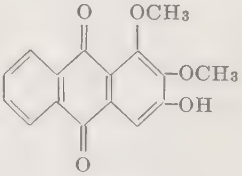
Purpurin

Anthragallol dimethyl ether A, $C_{16}H_{12}O_5$ (yellow needles, m.p. 218-220°), occurs in Indian madder, *Olenlandia umbellata,*[117] and has also been synthesized. It is isolated only by laborious purification after extracting the root with sulfurous acid.



Anthragallol dimethyl ether A

Anthragallol dimethyl ether B[117] (yellow needles, m.p. 230-232°) accompanies its isomeride (A) above in Indian madder, from which it may be separated by the greater solubility of the ammonium salt of the B isomeride in alcohol. Its synthesis also has been effected.



Ether B

Lapodin,[118] $C_{18}H_{16}O_5$ (yellow needles, m.p. 206°), which is provisionally regarded as 1,2-diethyl-anthragallol, occurs in *Rumex obtusifolius.*

Pseudopurpurin,[119] $C_{15}H_8O_7$ (red leaflets, m.p. 222-224°), accompanies purpurin and purpuroxanthin in madder and is distinguished from both of these by its being sparingly soluble in alcohol or benzene. On boiling with water pseudopurpurin loses carbon dioxide and passes into purpurin. The constitution of pseudopurpurin was established by its synthesis[120] from both anthraquinone-1,2-dihydroxy-3-carboxylic acid (alizarin carboxylic acid) and anthraquinone-1,4-dihydroxy-3-carboxylic acid (quinizarin carboxylic acid). Its lake with alumina possesses unusual brilliance and fastness to light and the superiority of natural madder lake [121] over the synthetic product is possibly due to the presence of this acid. It is best obtained from crude madder purpurin.

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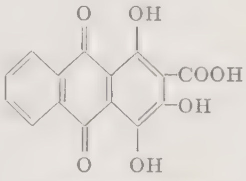
[117] A. G. Perkin, Hummel: *J. Chem. Soc.*, **63**, 1162 (1893); **67**, 823 (1895); A. G. Perkin, *Ibid.*, **91**, 2066 (1907).

[118] Hesse: *Ann.*, **309**, 52 (1899); Keegan: *Chem. News*, **114**, 74 (1916).

[119] Schützenberger: *Bull. soc. chim. (2)*, **4**, 12 (1865); Rosentiehl: *Ann. chim. (5)*, **13**, 256 (1878); Liebermann, Plath: *Ber.*, **10**, 1618 (1877).

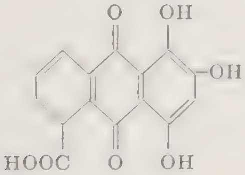
[120] D. R. P. 260,765, 272,301 (Farbenfabr. Bayer) Friedl., **11**, 591, 592; A. G. Perkin, Cope: *J. Chem. Soc.*, **65**, 847 (1894).



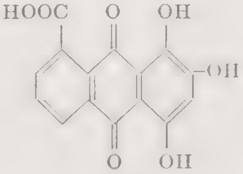
Pseudopurpurin

Pseudopurpurin occurs as a primoveroside, $C_{26}H_{26}O_{16}$ (yellow needles), in the root of the wild madder [122] *Rubia peregrina* and in *Galium verum* (yellow lady's bed straw) under the name galiosin.

Boletol. The stalks of *Boletus cyanescens* Bull., *B. luridus* Sch., *B. satanas* Lenz, *B. pachypus* Fr. and *B. lupinus Fr.* are colored red, and when the tissue is cut or broken the yellowish inner material exposed to the air quickly assumes a blue shade. Bertrand [123] was able to isolate the pigment responsible for the color of these fungi in the crystalline state; it is noteworthy that some years earlier Boehm [124] had obtained a similar compound, luridic acid, from *B. luridus,* but the identity of the two compounds may be doubted. The pigment boletol, isolated by Kögl [125] from *B. satanas* and *B. badius* Fr., has the empirical formula $C_{15}H_8O_7$, and crystallizes in red needles (m.p. 275-280°). Boletol gives a triacetate (yellow prisms, m.p. $> 300°$), but a pentaacetate (colorless prisms, m.p. 246°) may be obtained by reductive acetylation. A carboxyl group is also present and the carbon skeleton is indicated by the formation of anthracene on distillation with zinc dust, of purpurin on distillation with soda-lime and of hemimellitic acid on oxidation with hydrogen peroxide. Two structures (I) and (II) thus come into consideration:



(I)



(II)

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[121] Täuber: *Chemiker Ztg.*, 33, 1345 (1909); Cajar: *Oest. Chemik. Ztg. (2)*, 14, 173 (1911); C. Mayer: *Chemiker Ztg.*, 35, 1353 (1911).

[122] Hill: *Nature*, 134, 628 (1934); Hill, Richter: *J. Chem. Soc.*, **1936**, 1714.

[123] Bertrand: *Bull. soc. chim. (3)*, 27, 454 (1902).

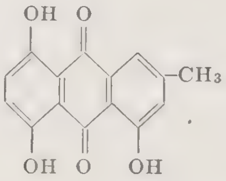
[124] Boehm: *Arch. Exp. Path. Pharmakol.*, 19, 60 (1885).

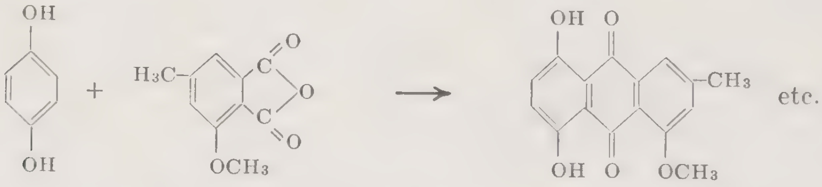
[125] Kögl, Deijs in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 1298.

The assumption of a blue color [126] by exposed tissue clearly depends upon the formation of a hydroxyanthradiquinone carboxylic acid, $C_{15}H_6O_7$, as 2 *p*-hydroxyl groups are oxidized to a quinone group. In the fungus this oxidation is effected by oxygen in association with oxidase enzymes, but it can also be effected in the laboratory with lead tetraacetate.

Synthetic experiments [127] starting from hemimellitic anhydride and 1,2,4-trimethoxybenzene tend to confirm the above formula but have not allowed any decision as to which is present in the natural pigment. Isolation is best effected by extracting the fungus with alcohol and further purifying the acid after precipitating as its lead salt. The yield varies widely, so that although 20 kg of *B. satanas* yield 1 g of the pigment, only 0.19 g of boletol was obtained from 70 kg of *B. badius.*

Helminthosporin, $C_{15}H_{10}O_5$, 4,5,8-trihydroxy-2-methylanthraquinone, forms chestnut-brown needles with a bronze luster (m.p. 226-227°) and is a metabolic product of *Helminthosporium gramineum* Rabenhorst when grown on Czapek-Dox solution with 5 per cent glucose. Proof of this constitution is afforded by its synthesis from hydroquinone and the methyl ether of γ-coccinic acid [128]:





Rubroglaucin,[129] $C_{16}H_{12}O_5$ (red needles, m.p. 180-181°), occurs in *Aspergillus glaucus.* Formerly stated to be 6-methoxy-1,4-dihydroxy-β-methylanthraquinone, rubroglaucin is, according to more recent investigation, not a single chemical entity.

Roseopurpurin, $C_{16}H_{12}O_6$, m.p. 285°, a product of *Penicillium roseopurpureum* Dierckx, is likewise a quinone related to β-methylanthracene.

[126] Kögl, Deijs: *Ann.*, 515, 10 (1934).

[127] Kögl, Deijs: *Ann.*, 515, 23 (1934).

[128] Raistrick, Robinson, Todd: *J. Chem. Soc.*, 1933, 488; Charles, Raistrick, Robinson, Todd: *Biochemical J.*, 27, 499 (1933); French P. 770,972 (I.C.I.), *Chem. Zentr.*, 1935, II, 1450; 4,5,8-trihydroxy-2-hydroxymethylanthraquinone is also produced.

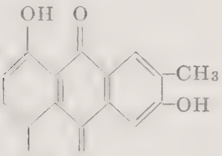
[129] Gould, Raistrick: *Biochemical J.*, 28, 1640 (1935); Raistrick, Robinson, Todd: *J. Chem. Soc.*, 1937, 80; Cruickshank, Raistrick, Robinson: *Ibid.*, 1938, 2056.

It contains one alcoholic and two phenolic groups; the last oxygen atom is present as a methoxy group which appears in 3-methoxybenzene-1:2:5-tricarboxylic acid on oxidizing with permanganate; the 5-carboxyl group must, on the analytical evidence, originate as a hydroxymethyl group; finally, the two phenolic groups are present, to judge from the variation in color with pH value, as a 1:3-dihydroxyanthraquinone system. Roseopurpurin is provisionally formulated as 4-methoxy-5:7-dihydroxy-2-hydroxymethylanthraquinone.[129a]

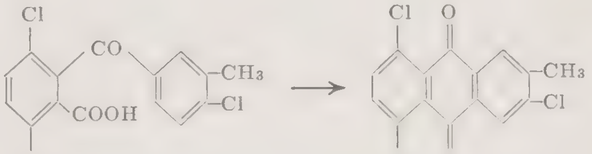
Citreorosein, $C_{15}H_{10}O_6$, m.p. 273-275°, is a metabolic product of *Penicillium citreoroseum* Dierckx. Its trimethyl ether is identical with the dimethyl ether of roseopurpurin, and since both pigments show similar color changes citreorosein is formulated as 4:5:7-trihydroxy-2-hydroxymethylanthraquinone.[129b]

Erythroglaucin.[130] When grown under controlled conditions differing from those under which rubroglaucin is formed, *Aspergillus glaucus* gives rise to a second pigment which is apparently a monomethyl ether of an α-methyltetrahydroanthraquinone, the exact constitution of which still awaits determination.

Rhabarberone (Isoemodin). This compound is found in rhubarb root *Rhizoma Rhei,* and crystallizes in yellow leaflets (m.p. 212°). It was formerly thought to be identical with emodin from aloes[131] but more recent work[132] has demonstrated that rhabarberone is 3,5,8-trihydroxy-2-methylanthraquinone (I); this has been synthesized through the trichloro-compound (II):



(I)



(II)

the orientation of which was in turn confirmed[133] by removing the methyl group to yield 3,5,8-(1,4,6-)trichloroanthraquinone, identical with an authentic specimen.

Morindone. This pigment occurs in the form of glycosides[134] in the roots of *Morinda citrifolia, Morinda tinctoria* (Soranjee) and *Morinda*

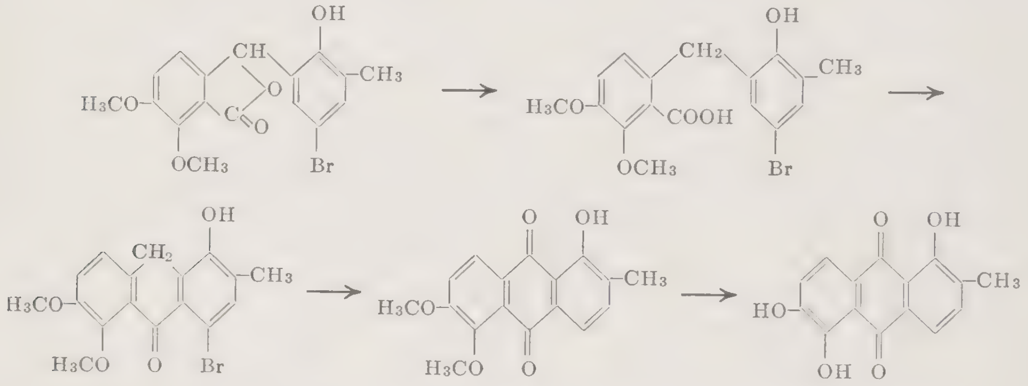
[129a] Posternak: *Helv. Chim. Acta,* 23, 1046 (1940).
[129b] Posternak, Jacobs: *Helv. Chim. Acta,* 23, 237 (1940).
[130] Raistrick: *Enzymologia,* 4, 76 (1937).
[131] Tutin, Clewer: *J. Chem. Soc.,* 99, 946 (1911); cf. Beilstein: Vol. **VIII,** p. 526.
[132] Keimatsu, Hirano: *J. pharm. Soc. Japan,* 49, 20 (1929); 51, 19 (1931).
[133] Egerer, Meyer: *Monatsh.,* 34, 69 (1913).
[134] Oesterle, Tisza: *Arch. Pharm.,* 245, 534 (1907); Simonsen: *J. Chem. Soc.,* 113, 766 (1918).

umbellata. That from the last-mentioned has the empirical formula $C_{26}H_{28}O_{14}$ (octaacetate, yellow needles, m.p. 246-248°) whereas the glycoside from the other two [135] has the formula $C_{27}H_{30}O_{15}$ (pale yellow needles, m.p. 205°). The same aglucone is thus probably associated with two sugar residues in the glycosides termed morindone:

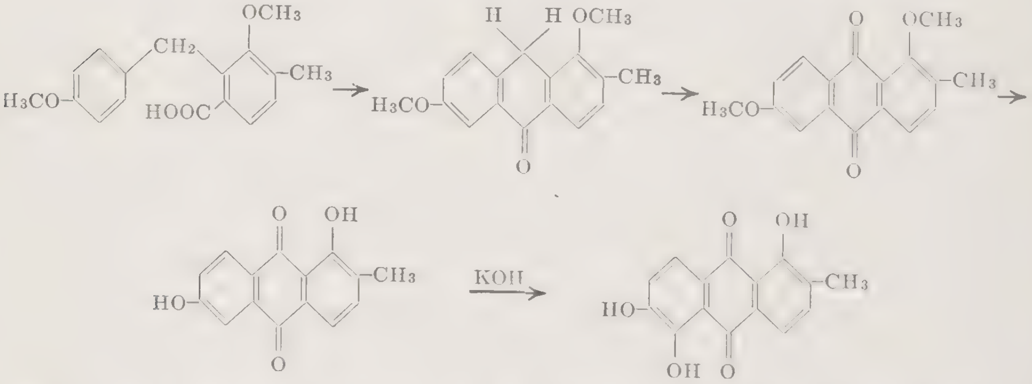
$$C_{27}H_{30}O_{15} + 2\ H_2O = C_{15}H_{10}O_5 + 2\ C_6H_{12}O_6$$
$$C_{26}H_{28}O_{14} + 2\ H_2O = C_{15}H_{10}O_5 + C_6H_{12}O_6 + C_5H_{10}O_5$$

Soranjee itself and Mang-Kondu, the root bark of *Morinda umbellata* [136] are also able to dye mordanted material.

Morindin,[137] $C_{15}H_{10}O_5$, forms orange-red needles (m.p. 281-282°), and is 1,5,6-trihydroxy-2-methylanthraquinone, as is confirmed by its synthesis from 3,4-dimethoxy-2′-hydroxy-3′-methyl-5′-bromodiphenylmethane-2-carboxylic acid (itself obtained from opianic acid and 5-bromo-2-cresol):


OH
CH
O
CH3
H3CO
C
O
OCH3
Br
OH
CH2
CH3
H3CO
COOH
OCH3
Br
OH
CH2
CH3
H3CO
H3CO
O
Br
O
OH
CH3
H3CO
H3CO
O
O
OH
CH3
HO
OH
O

or from 2′4-dimethoxy-3′-methyldiphenylmethane-6′-carboxylic acid:


OCH3
CH2
CH3
H3CO
HOOC
H
H
OCH3
CH3
H3CO
O
O
OCH3
CH3
H3CO
O
O
OH
CH3
HO
O
KOH
O
OH
CH3
HO
OH
O

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[135] A. G. Perkin, Hummel: *J. Chem. Soc.*, **65**, 851 (1894); A. G. and F. M. Perkin: *Proc. Chem. Soc. London*, **24**, 149 (1908).

[136] A. G. Perkin, Hummel: *J. Chem. Soc.*, **65**, 851 (1894).

[137] Anderson: *J. prakt. Chem. (1)*, **47**, 431 (1849); *Ann.*, **71**, 216 (1849); Thorpe, Grenall: *J. Chem. Soc.*, **51**, 56 (1887); Thorpe, Smith: *Ibid.*, **53**, 171 (1888); Oesterle, Tisza: *Arch. Pharm.*, **246**, 112 (1908); Jacobson, Adams: *J. Am. Chem. Soc.*, **46**, 2788 (1924); **47**, 283 (1925); Bhattacharya, Simonsen: *J. Indian Inst. Sci.* A, **10**, 6 (1927).

Morindone dissolves in concentrated sulfuric acid to a blue solution which becomes purple-red on standing. It is isolated by extracting the root-bark of *Morinda citrifolia* with alcohol and hydrolyzing the glycoside with dilute acid. Morindin, which is still used in India, dyes wool and silk an orange shade although the tint becomes more violet in the presence of soap; mordanted cotton is dyed a violet-brown color.

A trihydroxymethylanthraquinone methyl ether[138,139] which may be obtained in golden-yellow crystals (m.p. 216°) is found in Indian madder, in *Ventilago madraspatana*, and in *Morinda citrifolia* and *umbellata*. It is accompanied in the last two by morindanigrin[138] ($C_{16}H_{10}O_5$, yellow needles, m.p. 210°) and in *Morinda citrifolia* by another trihydroxymethylanthraquinone methyl ether, yellow needles (m.p. 172°).

The following related compounds also have been found in the root-bark of *Morinda umbellata*[138]: 1,3-Dihydroxy-6-methylanthraquinone [yellow needles, m.p. 269° (acetate, m.p. 165-167°)] identical with the synthetic product of Marchlewski[140]; a derivative of dimethylanthraquinone, $C_{16}H_{12}O_6$ [orange-red needles, m.p. 258° (acetyl compound, m.p. 129-130°)]; a hydroxymethylanthraquinone carboxylic acid, $C_{16}H_{10}O_5$ (yellow needles, m.p. 198-199°); *Morinda citrifolia* also contains[139,141] the pigments *Morindadiol*, $C_{15}H_{10}O_4$, 1,5-dihydroxy-methylanthraquinone, yellow needles (m.p. 244°), and *Soranjidiol*, $C_{15}H_{10}O_4$, 2,5-dihydroxy-6-methylanthraquinone (?), dark red-brown needles (m.p. 276°).

Emodin. This pigment occurs as a glycoside (frangulin) in the rhubarb-root,[142] *Cascara sagrada*, in buckthorn bark,[143] *Rhamnus frangula*, in *Rhamnus Purshianus* and other plants. The glycoside frangulin has the composition corresponding to $C_{21}H_{20}O_{10}$ (lemon-yellow needles, m.p. 239-241°), and on boiling with dilute acid undergoes fission into emodin and rhamnose:

$$C_{21}H_{20}O_{10} + H_2O = C_{15}H_{10}O_5 + C_6H_{12}O_6$$

Frangula bark contains also an anthranol glycoside, frangularoside,[144] $C_{21}H_{24}O_9$, which undergoes hydrolysis into rhamnose and frangularol (orange-yellow crystals, m.p. 301°). It is curious that analytical figures for frangularol agree with the formula $C_{15}H_{14}O_4$, although $C_{15}H_{14}O_5$ would be expected from the formula of the glycoside. The following related glycosides deserve brief mention: Shesterin[145] (pale yellow needles, m.p. 229-234°), which is hydrolyzed to a mixture of hexose and pentose and emodin anthranol, occurs in *Rhamnus cathartica.* Glucofrangulin,[146] $C_{27}H_{30}O_{14}$, from Frangula bark, forms an orange powder (m.p. 215°, after softening at 175°) and is composed of emodin, glucose and rhamnose. Polygonin,[147] $C_{21}H_{20}O_9$ (orange-yellow needles, m.p. 202-203°), from *Polygonum cuspidatum,* contains a hexose as the sugar constituent. Rhamnocathartin,[148] $C_{27}H_{30}O_{14}$ (yellow needles, m.p. 236°), from *Rhamnus cathartica* contains emodin, rhamnose and a hexose as structural units.

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[138] A. G. Perkin, Hummel: *J. Chem. Soc.*, 63, 1160 (1893); 65, 851 (1894).

[139] Oesterle: *Arch. Pharm.*, 245, 287 (1907); Oesterle, Tisza: *Ibid.*, 246, 150 (1908).

[140] Marchlewski: *J. Chem. Soc.*, 63, 1142 (1893).

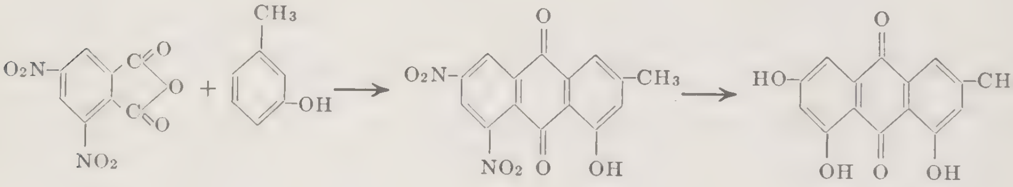
[141] Simonsen: *J. Chem. Soc.*, 117, 561 (1920).

[142] Literature: Beilstein: Vol. **VIII**, p. 520; "Isolation of a rhamnoglycoside," Sipple, King, Beal: *J. Am. Pharm. Assoc.*, 23, 205 (1924).

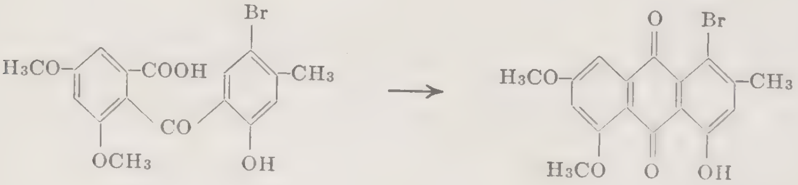
[143] Briedel, Charaux: *Compt. rend.*, 192, 1269 (1931). These workers mention a primary frangula-glycoside.

[144] Bridel, Charaux: *Compt. rend.*, 191, 1374 (1930); *Bull. soc. chim. biol.*, 17, 780, 793 (1935).

Emodin, $C_{15}H_{10}O_5$ (I), crystallizes in orange needles, dissolves in alkalies to red solutions and is 4,5,7-trihydroxy-2-methylanthraquinone. Emodin was synthesized first by Eder and Widmer[149] from *m*-cresol and 3,5-dinitrophthalic anhydride:


(I)

and later by Jacobson and Adams[150] from 2,4-dimethoxy(2′-hydroxy-4′-methyl-5′-bromo)-benzophenone-6-carboxylic acid:



Frangula or (alder) buckthorn bark is recommended as a source of emodin.[151]

Chrysophanic acid is accompanied by a methyl ether of emodin, 7-methoxy-4,5-dihydroxy-2-methylanthraquinone, $C_{16}H_{12}O_5$ [m.p. 199-

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[145] Waljaschko, Krassowski: *J. Russ. Phys. Chem. Soc.,* **40,** 1502 (1908); Krassowski: *Ibid.,* **40,** 1510 (1908).

[146] Casparis, Maeder: *Schweiz. Apoth. Z.,* **63,** 313 (1925).

[147] A. G. Perkin: *J. Chem. Soc.,* **67,** 1084 (1895).

[148] The Rhamnoxanthin of Waljaschko and Krassowski is identical with frangulin; cf. Bridel, Charaux: *Bull. soc. chim. biol.,* **15,** 648 (1933).

[149] Eder, Widmer: *Helv. Chim. Acta,* **6,** 966 (1923) (history and bibliography); Eder, Hauser: *Ibid.,* **8,** 126 (1925); D. R. P. 397,316 (Eder) Friedl., **14,** 1446. *See also* Anslow, Breen, Raistrick: *J. Chem. Soc.,* 1940, 427.

[150] Jacobson, Adams: *J. Am. Chem. Soc.,* **46,** 1312 (1924).

[151] Klein: "Handbuch der Pflanzenanalyse," **III,** 2, p. 1027; emodin from chrysarobin: Gardner: *J. Am. Pharm. Assoc.,* **28,** 143 (1939).

201° (203-204°)]. The latter is known as lichen chrysophanic acid,[152] physcion, parietin and parmel yellow, and occurs also in *Aspergillus ruber* and *glaucus* and in *Xanthoria parietina.*

Emodic acid and ω-*hydroxyemodin* are metabolic products of *Penicillium cyclopium* Westling. The former has the structure of 4:5:7-trihydroxyanthraquinone-2-carboxylic acid, since it is formed from Frangula emodin by oxidation with chromic acid. The tetracetate of ω-hydroxyemodin is oxidized to the triacetyl derivative of emodic acid, so that ω-hydroxyemodin is considered to be 4:5:7-trihydroxy-2-hydroxymethylanthraquinone and should be identical with citreorosein.[152a]

Penicilliopsin, the coloring principle of *Pencilliopsis clavariaeformis* Solms-Laubach, has the formula $C_{30}H_{24}O_8$ and m.p. 330° (decomp.). On heating, it gives Frangula emodin anthranol (4:5:7-trihydroxy-2-methylanthranol) and its relation to Frangula emodin is further emphasized by its behavior toward nitric acid. On atmospheric oxidation it gives oxypenicilliopsin, $C_{30}H_{20}O_9$, and this on irradiation passes into an isomeride which closely resembles hypericin.[152b]

Carviolin and *carviolacin* occur among the metabolic products of *Penicillium carmino-violaceum* Biourge. The first, $C_{16}H_{12}O_6$, m.p. 286°, is a quinone and also a triphenolic body; on reductive acetylation it gives leucocarviolin pentacetate. Carviolacin, $C_{20}H_{17}O_7$, m.p. 243°, is likewise a triphenolic anthraquinone and yields 2-methylanthracene on distillation with zinc dust.[152c]

Nephromin,[153] $C_{16}H_{12}O_6$ (ochre-red needles, m.p. 196°), occurs in the lichen *Nephromium lusitanicum,* indigenous to Newfoundland; it contains one oxygen atom (probably one hydroxyl group) more than physcion.

Rheochrysidine, $C_{16}H_{12}O_5$ (yellow needles, m.p. 206-207°), is the aglucone of rheochrysin, $C_{22}H_{22}O_{10}$ (yellow needles, m.p. 204°) (one of the constituents of rheopurgarin [154]) and is thought to be 4,5-dihydroxy-7-methoxy-2-methylanthraquinone.

Chrysarone,[155] $C_{15}H_{10}O_5$ (golden-yellow leaflets, m.p. 165°), occurs in the root of *Rheum rhaponticum* as glucochrysarone, $C_{21}H_{20}O_{10}$ (yellow crystals, no m.p. is given), which may be hydrolyzed to give chrysarone and glucose. The constitution of chrysarone is now established by synthesis [156] as 3,5,6-trihydroxy-2-methylanthraquinone:

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[152] Eder, Hauser: *Helv. Chim. Acta,* 8, 140 (1925); Raistrick: *Enzymologia,* 4, 76 (1937); Raistrick, Robinson, Todd: *J. Chem. Soc.,* 1937, 80.

[152a] Anslow, Breen, Raistrick: *Biochem. J.,* 34, 159 (1940).

[152b] Oxford, Raistrick: *Biochem. J.,* 34, 790 (1940).

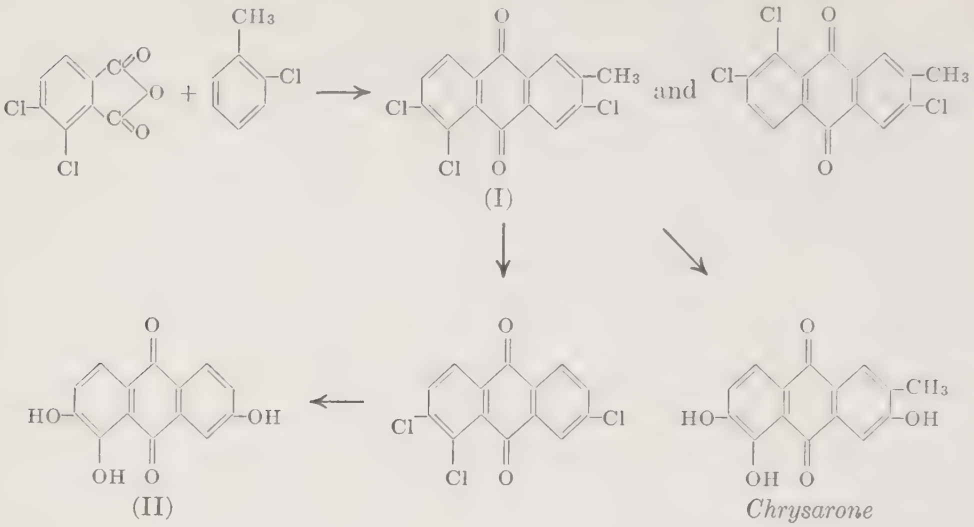
[152c] Hind: *Biochem. J.,* 34, 67 (1940).

[153] Bachmann: *Ber. deut. bot. Ges.,* 5, 192 (1887); Hesse: *J. prakt. Chem. (2),* 57, 443 (1898); 68, 52 (1903).

[154] Rheopurgarin is regarded as a mixture by Siegrist (Diss., Basel, 1932).

[155] Hesse: *J. prakt. Chem. (2),* 77, 347 (1908); *Ann.,* 309, 32 (1899).

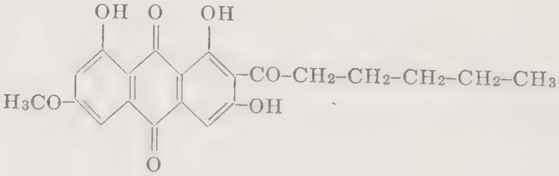
[156] Keimatsu, Hirano: *J. Pharm. Soc. Japan,* 49, 20 (1929); Keimatsu, Hirano, Tanabe: *Ibid.,* 49, 63 (1929).



The condensation of 3,4-dichlorphthalic anhydride with *o*-chlorotoluene may theoretically give rise to two anthraquinones, but the ambiguity was removed by converting (I) into a trihydroxyanthraquinone (II) and identifying this as 1,2,7-trihydroxyanthraquinone (anthrapurpurin).

Chrysarone is most conveniently obtained from Rhapontica root by extracting first with ether and then with acetone.

Solorinic acid[157] occurs in the thalli of *Solorina crocea*, a leafy lichen which flourishes in high Alpine regions as in the western Tyrol, St. Gotthard group, Engadine. It forms red leaflets (m.p. 203°) and has the composition corresponding to $C_{21}H_{20}O_7$ (although Hesse gives $C_{24}H_{22}O_8$). It forms a triacetyl derivative (m.p. 147°) and distillation with zinc dust gives 2-methylanthracene. Lastly, on being heated with hydriodic acid, capronic acid and the anthranol of 1,3,6,8-tetrahydroxyanthraquinone are obtained, so that the following structure is probable for solorinic acid:



Solorinic acid is obtained by extracting the thalli with ether.

Endocrocin.[158] This pigment, which has the formula $C_{16}H_{10}O_7$ and crystallizes in copper-red leaflets (m.p. 318°), was isolated from the Japanese lichen *Nephromopsis endocrocea*. It contains 2 keto-groups as a quinone, 3 phenolic hydroxyl groups and 1 carboxyl group, the last of which it may lose to yield Frangula emodin. From this and other degradative evidence the following structure is tentatively assigned:

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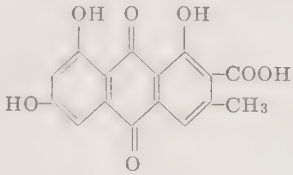
[157] Zopf: *Ann.*, **284,** 107 (1894); **364,** 273 (1909); Hesse: *J. prakt. Chem.* (2), **92,** 425 (1915); Koller, Russ: *Monatsh.*, **70,** 54 (1937).

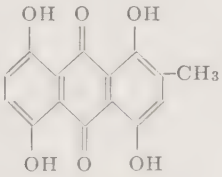
[158] Zopf: *Ann.*, **340,** 276 (1905); Asahina, Fuzikawa: *Ber.*, **68,** 1558 (1935).



Populnetin,[159] $C_{14}H_8O_6$ (m.p. 270-275°), occurs together with its glycoside populnin, $C_{20}H_{18}O_{11}$, in the dried petals of *Thespasia populnea*, the tulip. Populnetin is probably a tetrahydroxyanthraquinone. *Populus balsamifera* L. (Canada) produces a compound, $C_{15}H_{10}O_5$ (yellow needles, m.p. 218°), which is a trihydroxyanthraquinone derivative.[160]

Fallacin,[161] $C_{17}H_{14}O_6$ (yellow-brown needles, m.p. 240-241°), is obtained from *Xanthovia fallax* (Hepp.). Its constitution has been only partly elucidated, and the formulas $C_{14}H_2O_2(CH_3)_2(OH)_3(OCH_3)$, or more probably $C_{14}H_3O_2(CH_3)(CH_2OH)(OH)_2(OCH_3)$ embody the known facts.

Cynodontin,[162] $C_{15}H_{10}O_6$, is contained in the mycelium of *Helminthosporium cynodontis* Marignoni. It forms brown needles (m.p. 260°) and is identical with 1,4,5,8-tetrahydroxy-2-methylanthraquinone:



Catenarin,[163] $C_{15}H_{10}O_6$ (red plates, m.p. 246°), has been found in the mycelia of *Helminthosporium catenarium*, *H. tritici vulgaris* and *H. velutinum.* Catenarin is a β-hydroxymethyl-1,4,5-trihydroxyanthraquinone and may be oxidized to the corresponding β-hydroxymethyl-1,4,5,8-tetrahydroxyanthraquinone, $C_{15}H_{10}O_7$ (brownish leaflets, m.p. 320°).

Tritisporin,[163] $C_{15}H_{10}O_7$ (red-brown needles, m.p. 260-262°), isolated from the mycelium of *Helminthosporium tritici vulgaris,* is a 6- or 7-hydroxymethyl-1,3,5,8-tetrahydroxyanthraquinone.

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[159] Neelakantam, Seshadri: *Chem. Zentr.*, **1938**, II, 3813.

[160] Goris, Canal: *Bull. Soc. chim. (5)*, **3**, 1992 (1936).

[161] Asano, Fuziwara: *J. Pharm. Soc. Japan*, **56**, 185 (1936).

[162] Raistrick, Robinson, Todd: *Biochemical J.*, **28**, 559 (1934).

[163] Raistrick, Robinson, Todd: *Ibid.*, **28**, 559 (1934); cf. also Marriott, Robinson: *J. Chem. Soc.*, **1934**, 1631; and Charlesworth, Robinson: *Ibid.*, **1934**, 1531.

Pigments of Dermocybe sanguinea. This somewhat rare fungus occurring in the pine forests of Germany contains two pigments[164]:

(1) A red-yellow pigment, $C_{15}H_{10}O_5$ (needles, m.p. 253-254°), identical with Frangula emodin (q.v.), 4,5,7-trihydroxy-2-methylanthraquinone,[165] and

(2) **Dermocybin,** a red coloring principle with the formula $C_{16}H_{12}O_7$ (needles, m.p. 228-229°), which is a tetrahydroxymethoxy-2-methylanthraquinone, the orientation of the substituents having not yet been determined. The characteristic groups are indicated, however, by the formation of 2-methylanthracene on distillation with zinc dust, of a tetraacetate, and by the Zeisel reaction. The methoxy group may be hydrolyzed also with concentrated sulfuric acid at 150°, the resulting pentahydroxy-2-methylanthraquinone crystallizing in small red rhomboids, m.p. 289°. Dermocybin dissolves in concentrated sulfuric acid to violet, and in caustic alkalies to red-violet, solutions.

The pigments were isolated by exhaustively extracting the dried fungus with alcohol and evaporating the extract to dryness. The residue is taken up in ammonia, the crude pigments precipitated with acid and redissolved in pyridine. The yellow pigment is precipitated on adding water (yield 3 per cent of the dried fungus) and the red pigment is thrown down when the solution is made acid (yield 0.2-0.4 per cent). Dermocybin gives violet-red dyeings on wool mordanted with chromium. The pigment of the fungus *Dermocybe cinnabarina* is similar to dermocybin.

Graebeite.[166] A bright red efflorescence was observed by Treibs in the fractures of rock specimens obtained from blocks of slate clay from the Ölsnitz (Saxony) mine, lying 291 meters beneath the surface in a fault at the junction of the coal and lower new red sandstone. This is an organic mineral and consists of two pigments, graebeite *a* and graebeite *b*. The first is a compound, $C_{18}H_{14}O_8$ or $C_{17}H_{14}O_8$ (m.p. 250°), which is closely related to the polyhydroxyanthraquinones; on subliming it, a hexahydroxyanthraquinone, $C_{14}H_8O_8$ (orange-red needles, m.p. 245°), of unknown constitution is obtained.

Rhodocladonic acid.[167] The pigment is found in the lichen *Cladonia fimbriata, e.g.,* in Wildbad (Württemberg). It forms ruby-red crystals (m.p. 210-230°) and analytical figures indicate a formula $C_{17}H_{22}O_9$ or $C_{15}H_{20}O_8$. This partial structural formula is tentatively suggested:

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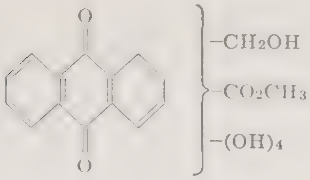
[164] Kögl, Postowsky: *Ann.,* 444, 1 (1925).

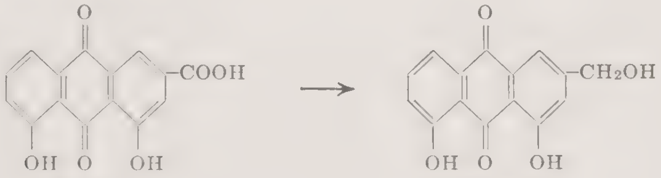
[165] Synthesized by Eder and Widmer: *Helv. Chim. Acta.,* 6, 966 (1923); by Eder and Hauser: *Ibid.,* 8, 126 (1925).

[166] Treibs, Steinmetz: *Ann.,* 506, 171 (1933).

[167] Zopf: *Ber. deut. bot. Ges.,* 26, 51 (1908); Hesse: *J. prakt. Chem. (2),* 83, 22 (1911): 92, 425 (1915); Koller, Hamburg: *Monatsh.,* 68, 202 (1936).

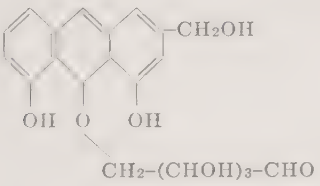


Aloin. The pigment,[168] which is also termed barbaloin, is found in aloe or aloes, the dried juice of the aloe plants (*Aloe spicata, arborescenz, linguaformis, lucida, socotrina, vulgaris,* etc.). Earlier analyses indicated formulas such as $C_{21}H_{20}O_9$, $C_{20}H_{18}O_9$,[169] $C_{20}H_{20}O_8$,[170] or $C_{16}H_{18}O_7$.[171] Aloin forms pale yellow needles (m.p. 147°). The following facts appear to be significant in the elucidation of its constitutional formula. Aloin undergoes hydrolysis to give aloe-emodin and arabinose although in very poor yield, as the conditions are not those normally employed for simple fission of a glycoside. Aloe emodin, $C_{15}H_{10}O_5$ (orange-yellow needles, m.p. 224-225°), is 4,5-dihydroxy-2-hydroxymethylanthraquinone[172] and has been prepared from rhein[173] by reducing the carboxyl group of the latter through the acid chloride and aldehyde:

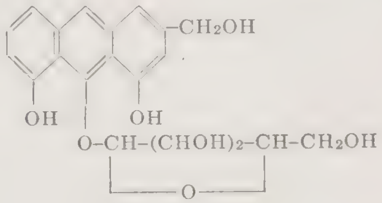


Rhein *Aloe-emodin*

With borax solution[174] however it has been claimed that aloin undergoes fission into 4,5-dihydroxy-2-hydroxymethylanthran-10-ol and arabinose, and on the basis of this observation Hauser[175] and also Rosenthaler[176] have proposed the following very similar structures:



(*Hauser*)



(*Rosenthaler*)

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[168] Older literature: Beilstein: Vol. **VIII**, p. 524.

[169] Léger: *Ann. chim. (9),* **6,** 318 (1916); **8,** 265 (1917); *J. pharm. chim. (8),* **18,** 25 (1933).

[170] Hauser: *Pharm. Helv. Acta,* **6,** 79 (1931).

[171] Robinson, Simonsen: *J. Chem. Soc.,* **95,** 1085 (1909); Gibson, Simonsen: *Ibid.,* **1930,** 553; Cahn, Simonsen: *Ibid.,* **1932,** 2573.

[172] Léger: *J. pharm. chim. (7),* **4,** 241 (1911); Oesterle: *Arch. Pharm.,* **250,** 304 (1912).

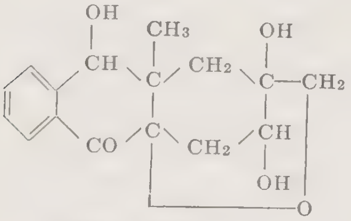
[173] Mitter, Banerjee: *J. Indian Chem. Soc.,* **9,** 375 (1932).

[174] Cf. McDonnell, Gardner: *J. Am. Chem. Soc.,* **56,** 1246 (1934).

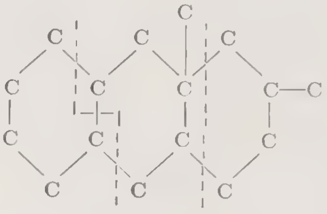
[175] Hauser: *Pharm. Acta Helv.,* **6,** 79 (1931).

[176] Rosenthaler: *Schweiz. Apoth. Z.,* **69,** 255 (1931); *Pharm. Acta Helv.,* 6, 115 (1931); *Arch. Pharm.,* **270,** 214 (1932); *Pharm. Acta Helv.,* **7,** 19 (1932); **9,** 9 (1934).

On the other hand Cahn and Simonsen [177] have pointed out that these formulas are not in complete agreement with the experimental facts; the amount of arabinose formed is so small and the conditions so abnormal that they suggest the degradation is not merely a hydrolytic one. They accordingly revert to Tilden's formula, $C_{16}H_{18}O_7$, and formulate aloin as follows:



Tilden's formula



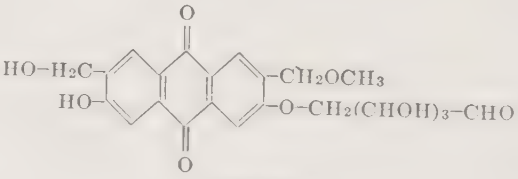
(I)

The formation of arabinose would then follow rupture across the dotted lines. Moreover this structure of barbaloin is easily derived from one hexose and two isoprene units (I). Rosenthaler [178] and Gardner [179] find objection to this formulation, the latter on the ground, among others, that an aloe-emodin-*d*-arabinoside should not reduce Fehling's solution as aloin does. Aloin is obtained [180] by extracting varieties of aloes with methanol.

β-Barbaloin,[179] which accumulates in the mother-liquor after removing barbaloin, is probably a stereoisomeride.

Isobarbaloin was formerly regarded as the 9-anthranol glycoside, but in view of the assertions of Cahn and Simonsen this formulation stands in need of confirmation. Gardner [181] is of the opinion in this connection that the difference between barbaloin and isobarbaloin does not consist in structural differences in the anthracene nuclei.

Nataloin, from Natal aloes, is according to Léger [182] a mixture of two compounds, nataloin and homonataloin, for which the following structures are suggested:



Nataloin

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[177] Cahn, Simonsen: *J. Chem. Soc.*, **1932**, 2573.

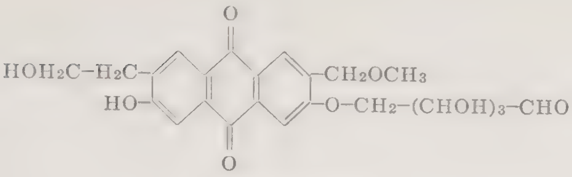
[178] Rosenthaler: *Pharm. Acta Helv.*, **9**, 9 (1934).

[179] Gardner, McDonnell, Wiegand: *J. Am. Chem. Soc.*, **57**, 1074 (1935); Foster, Gardner: *Ibid.*, **58**, 597 (1936).

[180] Léger: *Ann. chim. (9)*, **6**, 318 (1916); Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 992 (Rosenthaler: "Anthracenglucoside").

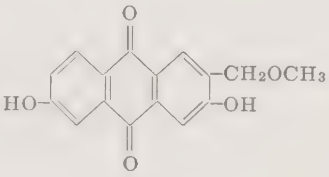
[181] Gardner, Joseph: *J. Am. Pharm. Assoc.*, **26**, 794 (1937).

[182] Léger: *Ann. chim. (9)*, **6**, 318 (1916); *(9)*, **8**, 265 (1917).



Homonataloin

but in view of the above findings these structures are subject to revision. With sodium peroxide a compound which should be a methyl nataloe-emodin, 2-methoxymethyl-3,6-dihydroxyanthraquinone (orange-red needles, m.p. 238°) (I) is obtained:



(I)

Blastenin,[183] fragilin,[184] hymenorhodin,[185] placodin,[186] and rhodophyscin [187] are other pigments stated to be anthraquinone derivatives which have been isolated from lichens.

Anchusin, $C_{30}H_{36}O_9$, is a constituent of alkanet root (*Anchusa tinctoria*). It is clearly a substituted anthraquinone derivative, as it yields 2-methylanthracene with zinc dust. Anchusin contains one alcoholic residue and two phenolic groups.[187a]

Anthracene Insect Pigments

Cochineal. The female of a scale-insect, *Coccus cacti,* which flourishes on the cactus species *Nopalea coccinellifera* in Mexico and Central America contains a red pigment, carminic acid, which found wide application until it was replaced by the cheaper red azo dyestuffs. About 140,000 insects weigh 1 kg and a plantation of 3 hectares (100 $m.^2$) yields about 300 kg of cochineal. This product contains approximately 10 per cent of pigment obtained by grinding the insect mass after subjecting it to steam or dry heat. The pigment dyes mordanted wool and silk,[188] the tin lake on wool giving a beautiful scarlet-red color which is quite fast to light and washing.

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[183] Senft: *Z. allg. Öster. Apoth. Ver.*, **52**, 165 (1914); Hesse: *J. prakt. Chem. (2)*, **58**, 465 (1899); *(2)*, **63**, 522 (1901).

[184] Zopf: *Ann.*, **300**, 322 (1898); **340**, 276 (1905).

[185] Zopf: *Ann.*, **346**, 82 (1906).

[186] Zopf: *Ann.*, **288**, 38 (1895).

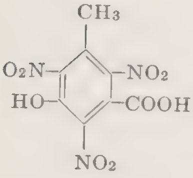
[187] Senft: *Z. allg. Öster. Apoth. Ver.*, **52**, 165 (1914); Zopf: *Ann.*, **340**, 276 (1905).

[187a] Majumdar, Chakravarty: *J. Indian Chem. Soc.*, **17**, 272 (1940).

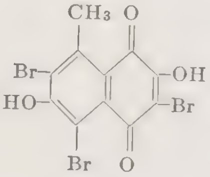
[188] Composition of precipitates with metal salts: Guggiari: *Ber.*, **45**, 2442 (1912).

Carminic acid, obtained[189] by purification through its lead salt, forms red needles which darken at 130° and carbonize at 250°. Carmin[190] is prepared by extracting cochineal with water and precipitating an aluminum salt of carminic acid (which still contains nitrogenous material) with alum. Carmin is used as a water-color, in rouge, to stain microscopic preparations and to color foodstuffs.

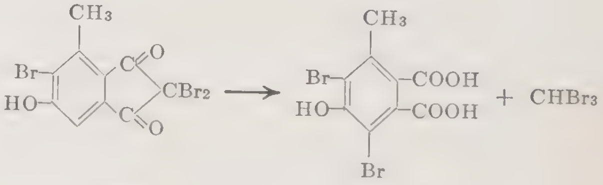
The molecular formula $C_{22}H_{22}O_{13}$ proposed by Liebermann[191] has been altered by Dimroth[192] to $C_{22}H_{20}O_{13}$. Oxidation[193] with nitric acid yielded nitrococcussic acid, which was shown by synthesis[194] to be a trinitrocresotinic acid of structure (I). By the action of bromine[195] two degradation products were obtained: β-bromocarmin, $C_{11}H_5O_4Br_3$ [orange needles, m.p. 238° (v. Miller and Rohde)], believed to be a naphthoquinone derivative[196] (II), as naphthalene was obtained by distillation with zinc dust; and α-bromocarmin, $C_{10}H_4O_3Br_3$ (colorless needles, m.p. 247-248°), which is an indane-dione derivative (III), as it undergoes fission, on warming with sodium carbonate solution, into bromoform and methyldibromohydroxyphthalic acid[197] (Will, Leymann):



(I)



(II)



(III)

Oxidation of carminic acid by hydrogen peroxide in the presence of catalysts (preferably cobalt sulfate) yields 8-methyl-2,6-dihydroxy-1,4-naphthoquinone-3,5-dicarboxylic acid (IV), which is converted by potassium permanganate into carminazarin,[198] $C_{12}H_8O_7$ (red needles, m.p.

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[189] Schunck, Marchlewski: *Ber.*, **27**, 2979 (1894); v. Miller, Rohde: *Ber.*, **30**, 1762 (1897); Dimroth: *Ann.*, **399**, 1 (1913).

[190] Liebermann: *Ber.*, **18**, 1969 (1885); Frey: "Zur Kenntnis des Carmins und der Neocarminsäure," Diss. Techn. Hochschule, Zurich, 1931; Driessen: *Rev. gén. mat. color.*, **40**, 416 (1936); Karr: *Textile Col.*, **58**, 672 (1936).

[191] Liebermann, Höring, Wildermann: *Ber.*, **33**, 149 (1900); Review of earlier literature: v. Miller, Rohde: *Ber.*, **26**, 2647 (1893); further work partly of historical value only: Liebermann: *Ber.*, **18**, 1969, 1975 (1885); **31**, 2079 (1898); Landau: *Ber.*, **33**, 2442, 2446 (1900); Liebermann, Landau: *Ber.*, **34**, 2153 (1901); Liebermann, Lindenbaum: *Ber.*, **35**, 2910 (1902); v. Miller, Rohde: *Ber.*, **30**, 1759 (1897).

[192] Dimroth, Kämmerer: *Ber.*, **53**, 471 (1920).

[193] de la Rue: *Ann.*, **64**, 1 (1848); Liebermann, v. Dorp., *Ann.*, **163**, 97 (1872).

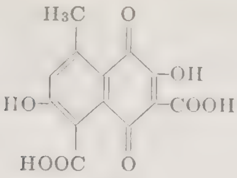
[194] v. Kostanecki, Niementowski: *Ber.*, **18**, 250 (1885).

[195] Will, Leymann: *Ber.*, **18**, 3180 (1885).

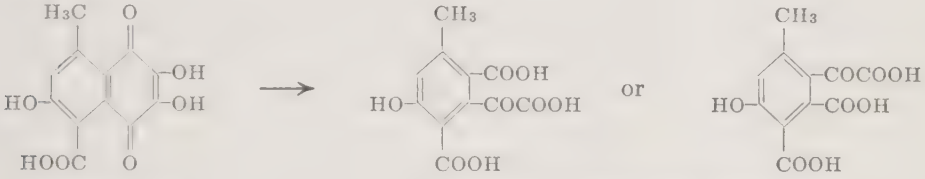
[196] v. Miller, Rohde: *Ber.*, **26**, 2647 (1893); cf. the divergent interpretation of Liebermann. Voswinckel: *Ber.*, **30**, 1731 (1897) and the reply of Rohde, Dorfmüller: *Ber.*, **43**, 1363 (1910); Dimroth: *Ann.*, **399**, 1 (1913).

[197] v. Miller, Rohde: *Ber.*, **26**, 2647 (1893); cf. Zincke, Gerland: *Ber.*, **20**, 3216 (1887); **21**, 2379 (1888).

[198] Dimroth: *Ber.*, **42**, 1611, 1735 (1909).

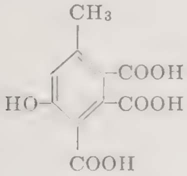

(IV)

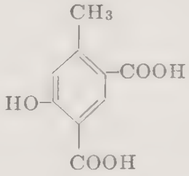
240-250°). The same product may be obtained from carminic acid using potassium permanganate in sulfuric acid at 0°:

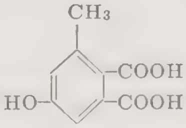

Carminazarin

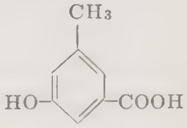
Cresotinic-glyoxylic-dicarboxylic acid,
(colorless needless, m.p. 230°)

To cochenillic acid [199] (colorless needles, m.p. 224-225°), obtained by oxidizing carminic acid with potassium persulfate, is assigned constitution (I), as it may be degraded to α- or β-coccinic acid (II), (III), or to cresotinic acid (IV), according to the experimental conditions:

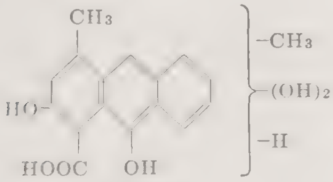

(I)

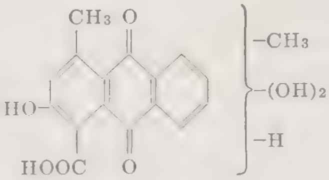

(II)


(III)


(IV)

Fusion of carminic acid with potash yields coccinin,[200] $C_{17}H_{14}O_6$, which crystallizes in straw-yellow needles. Coccinin dissolves in alkalies to a yellow solution, which gradually becomes violet as the coccinin is irreversibly converted into coccinone, $C_{17}H_{12}O_7$. These compounds are formulated by Dimroth thus:


Coccinin


Coccinone

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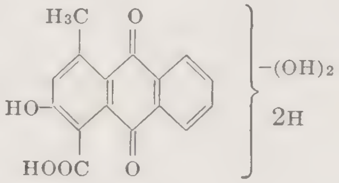
[199] Liebermann, Voswinckel: *Ber.*, **30**, 688, 1731 (1897); Dimroth: *Ber.*, **43**, 1387 (1910); cf. C. Liebermann, H. Liebermann: *Ber.*, **42**, 1922 (1909). Unsuccessful attempts to synthesize: Schleussner, Voswinckel: *Ann.*, **422**, 111 (1921); Synthesis of β- and γ-coccinic acid: Meldrumsen, Vaidyanathansen: *Chem. Zentr.*, **1935**, II. 1370.

[200] Hlasiwetz, Grabowski: *Ann.*, **141**, 329 (1867); Dimroth: *Ann.*, **399**, 1 (1913).

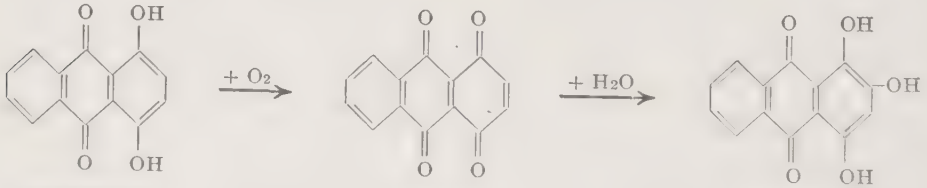
In agreement with these structures coccinin yields a tetracetyl compound, whereas coccinone gives only a triacetyl derivative. The two hydroxyl groups which in Dimroth's formula are not assigned definite positions are probably placed on the 1,4-carbon atoms, bearing in mind the evidence of carminic acid itself. The carboxyl groups may be removed to give a dimethyltrihydroxyanthraquinone. It may be remarked that in agreement with these partial formulas carminic acid, on distillation with zinc dust, gives a small yield of anthracene and α-methylanthracene,[201] and with sulfuric acid [202] a compound, $C_{16}H_{10}O_7$ (needles, m.p. 305°), which is probably a methyltrihydroxyanthraquinone carboxylic acid (I), is obtained:



(I)

Bamberger and Praetorius [203] correlated the oxidative formation of naphthoquinones from carmin with the presence of a hydroxyl group in the anthraquinone nucleus and quoted a number of analogous instances.

On reducing carminic acid with zinc dust and acetic acid [204] a leuco-compound is formed, which is oxidized in alkaline solution to a new pigment, desoxycarminic acid, which differs from carminic acid in containing one less oxygen atom. This compound, like quinizarin, gives a diquinone which may be reconverted into carminic acid just as quinizarin diquinone is converted into purpurin.[205]



quinizarin *quinizarin diquinone* *purpurin*

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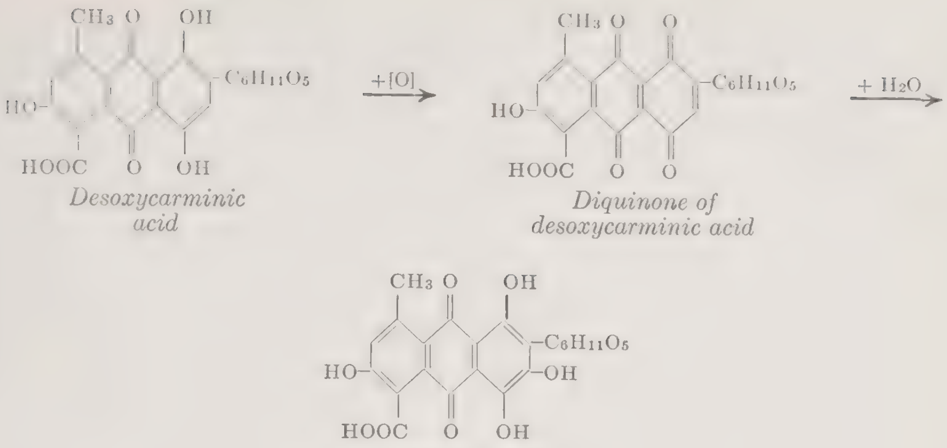
[201] Cf. also Fürth: *Ber.*, **16**, 2169 (1883).

[202] The ruficoccin of Liebermann and van Dorp obtained by the action of sulfuric acid on carminic acid is, according to C. and H. Liebermann [*Ber.*, **47**, 1213 (1914)], a mixture of this acid with its decarboxylated derivative.

[203] Bamberger, Praetorius: *Monatsh.*, **23**, 688 (1902); Dimroth, Schultze: *Ann.*, **411**, 339 (1916); Scholl, Zinke: *Ber.*, **51**, 1419 (1918); **52**, 1142 (1919).

[204] Dimroth, Kämmerer: *Ber.*, **53**, 471 (1920).

[205] Dimroth, Schultze: *Ann.*, **411**, 345 (1919).



Desoxycarminic acid

Diquinone of desoxycarminic acid

Carminic acid[206]

Of the residue, $C_6H_{11}O_5$, it is known only that it contains four hydroxyl groups which can be aceylated and that, although it is essentially a sugar group,[207] it is not bound to the anthraquinone as a glycoside. Asymmetric carbon atoms are present, as carminic acid is dextro-rotatory.

Frey,[208] noting the small yield obtained in the preparation of carminic acid, found in the mother-liquors a second pigment, neocarminic acid, the examination of which he describes.

Kermes. Kermes consists of the dried insect, *Coccus ilici,* which flourishes on the holm-oak (Ilex) and the evergreen or alkermes-oak (*Quercus coccifera*) in Spain, Portugal and Morocco. The dyestuff Venetian scarlet was prepared from kermes; but kermes, which was known to the ancients, was displaced by cochineal. Kermesic acid, as Dimroth termed the pigment, was first prepared in the pure state by Heisse. According to Dimroth,[209] who followed the procedure of Heisse, the crude material is first freed from a wax, cerotic ceryl ester,[210] by extracting with ether; then the pigment, which is present as a salt, is liberated by ethereal hydrochloric acid and taken up in ether. It is purified through the sodium salt and again precipitated with acid. Five kg of crude material yield approximately 50-55 grams of pigment.

Kermesic acid, $C_{18}H_{12}O_9$, forms brick-red needles and dissolves in water to yellow-red, and in sulfuric acid to violet-red, solutions. It dyes

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[206] Older formulas of carminic acid: Liebermann, Voswinckel: *Ber.,* **30,** 1731 (1897); Radulescu: *Bul. Soc. Stiinte Bucarest,* 21, 32 (1912). Considerations unfavorable to anthraquinone formulas: H. and C. Liebermann: *Ber.,* 47, 1213 (1914); reply, Dimroth, Kämmerer: *Ber.,* 53, 471 (1920). The formulas given by Justin-Mueller *Bull. soc. chim. (4),* **39,** 791 (1926)] and wrongly ascribed to Dimroth, are erroneous.

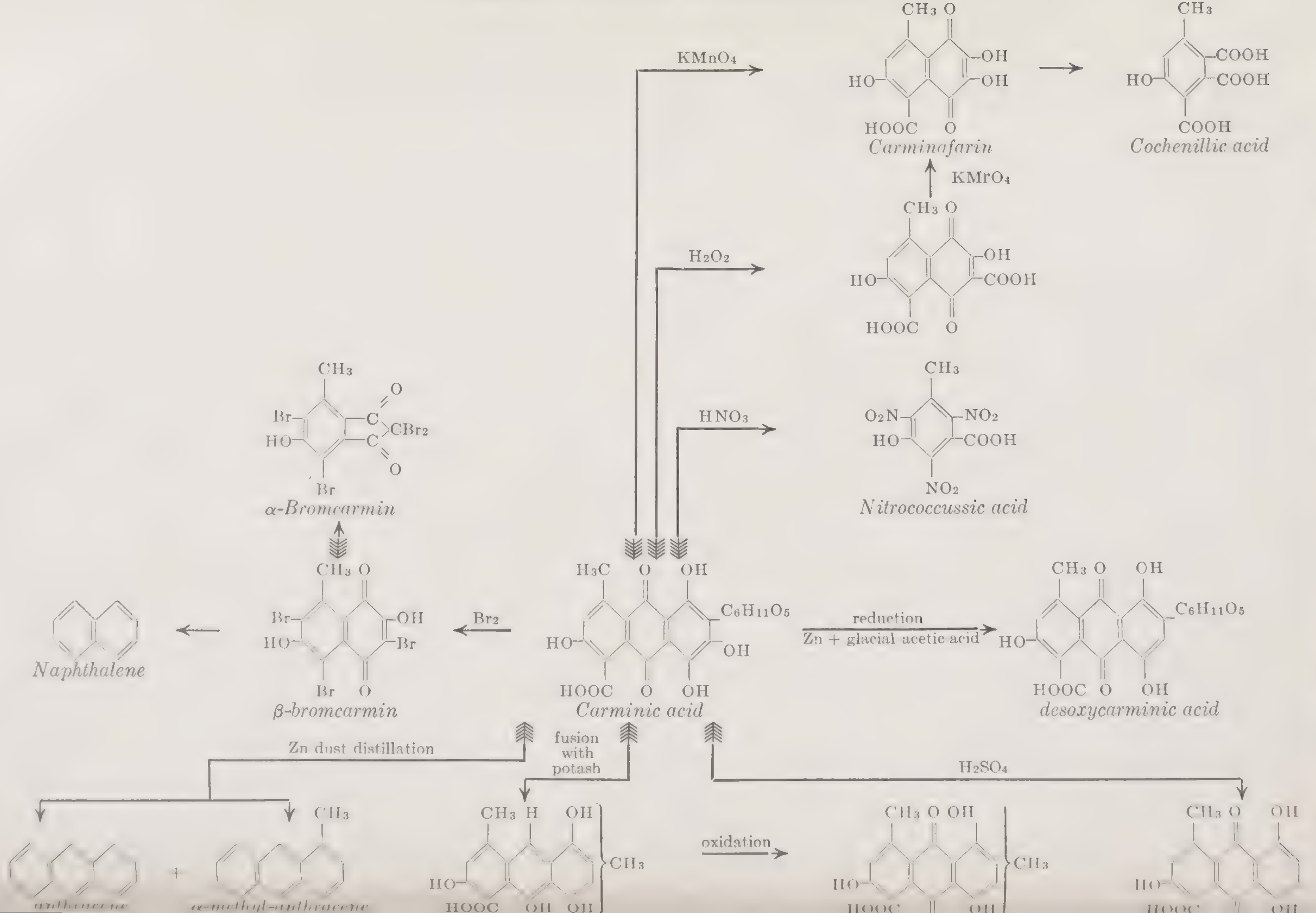
[207] Miyagawa: *Chem. Zentr.,* **1937,** I, 885.

[208] Frey: "Zur Kenntnis des Carmins und der Neocarminsäure," Diss., Technische Hochschule, Zurich, 1931.

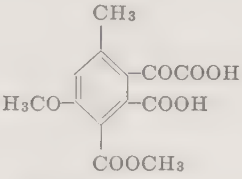
[209] Dimroth: *Ber.,* **43,** 1387 (1910).

[210] Dimroth, Scheurer: *Ann.,* **399,** 60 (1913).

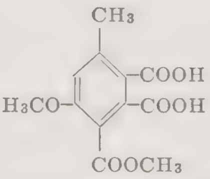
Degradation Products of Carminic Acid


KMnO₄
H₂O₂
HNO₃
Carminafarin
Cochenillic acid
KMrO₄
Nitrococcussic acid
α-Bromcarmin
Br₂
β-bromcarmin
Naphthalene
Carminic acid
reduction
Zn + glacial acetic acid
desoxycarminic acid
Zn dust distillation
fusion with potash
H₂SO₄
oxidation
anthracene
α-methyl-anthracene

wool orange-red from an acid bath and gives scarlet-red dyeings which are less blue than those of carmin on a tin mordant. The constitution of kermesic acid[211] is deduced from the following observations. It contains four hydroxyl groups, which may be acetylated, and oxidation yields nitrococcusic acid, also obtained from cochineal. Methylation with dimethyl sulfate gives a trimethyl derivative, by oxidation of which with potassium permanganate the methyl esters of two acids are obtained:

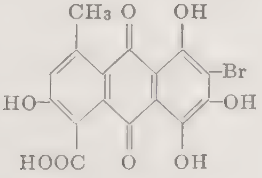


Colorless tablets
(m.p. 108-109°)

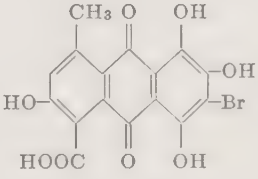


Methyl ether of cochinillic acid
methyl ester

Again, bromination in acetic acid yields the α-bromcarmin obtained from carminic acid, whereas, when the bromination is effected in boiling acetic acid, a monobromcoccin, $C_{16}H_9O_8Br$ (fine needles, m.p. 259-260°), is obtained, which may be oxidized by hydrogen peroxide to cochenillic acid. Distillation with zinc dust gives α-methylanthracene. Monobromcoccin therefore possesses one of the two following constitutions:



(A)



(B)

The left-hand benzene ring is thus that which appears on degradation as cochenillic acid; the orientation of the three hydroxyl groups in the right-hand nucleus is probable from its properties as a pigment, as otherwise derivatives of anthragallol, which would behave differently as dyes, would be observed. Now, as kermesic acid differs from bromococcin only in containing the group C_2H_3O in place of Br, and as this residue can be resolved only into CH_3CO— or CH_2—CHO, the second of which is excluded because no aldehyde properties are observed, kermesic acid[212] must correspond to formula (I) or (II):

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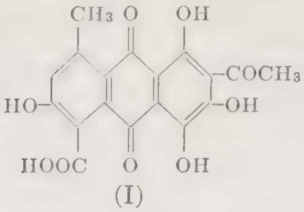
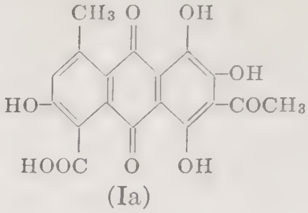
[211] Dimroth: *Ber.*, **43**, 1387 (1910); Dimroth, Scheurer: *Ann.*, **399**, 43 (1913); Dimroth, Fick: *Ann.*, 411, 315 (1916).

[212] For the varying effect of the acetyl group in anthraquinone compounds on their color, see F. Mayer, Stark, Schön: *Ber.*, **65**, 1333 (1932).

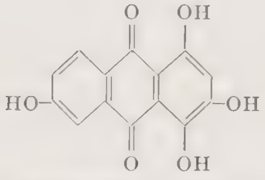


(I)

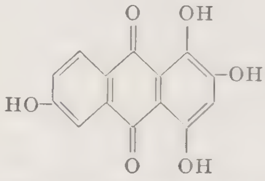


(Ia)

Carminic acid and decarboxykermesic acid possess the same color properties in alkaline solution as does hydroxyanthrapurpurin, as distinct from hydroxyflavopurpurin:



Hydroxyanthrapurpurin



Hydroxyflavopurpurin

so that formula (A) is more probable for bromococcin and (I) for kermesic acid.

Further support for the anthraquinone formulation is provided by the formation of 1-methyl-3,5,8-trihydroxyanthraquinone (red needles, m.p. 260°) on reducing kermesic acid with zinc dust. This compound has been obtained from 1-methyl-3,5,7,8-tetrahydroxyanthraquinone,[213] and also synthetically from β-coccinic anhydride and hydroquinone (page 145).

Crude kermes contains, in addition to kermesic acid, a second pigment,[214] flavokermesic acid (3 g from 5 kg of kermes), the two being separated by taking advantage of the different solubilities of their sodium salts in sodium acetate solution. Flavokermesic acid, $C_{13}H_8O_6$, forms yellow needles and dissolves in concentrated sulfuric acid to a red-brown solution. Golden-yellow dyeings are obtained from acid baths, and on tin mordants a dull orange shade is produced.

Lac-dye. This coloring matter is contained in stick- or gum-lac; the lac is the solidified exudation[215] of the insect *Coccus laccae* growing on various trees found in the East Indies, Ceylon and the Molucca Islands. The dye is obtained by extracting stick-lac with water and sodium carbonate solution and precipitating with alum or lime, in which forms it appears in commerce under the names lac-dye or lac-lac. Lac-dye has been prepared for centuries past in the East Indies,[216] as it yields similar

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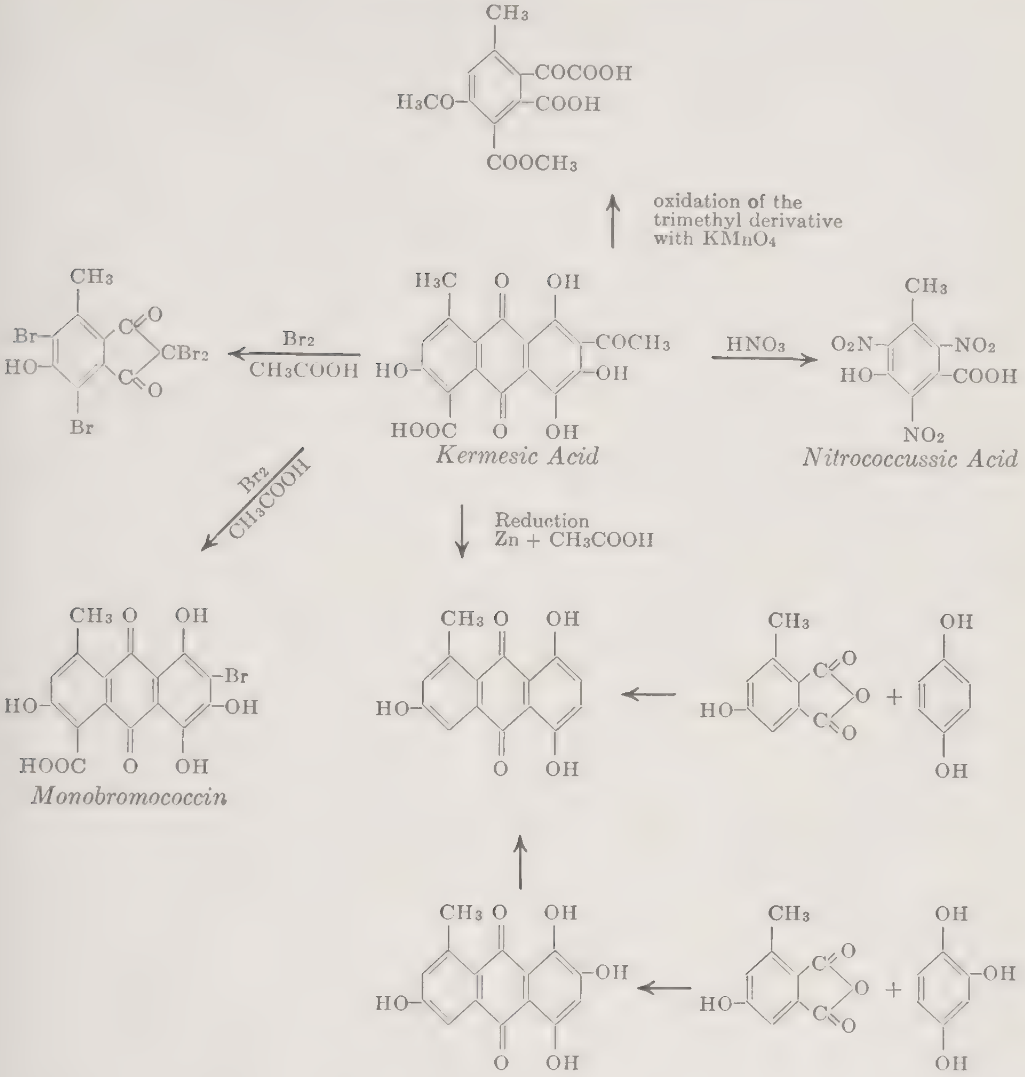
[213] Dimroth, Fick: *Ann.*, **411**, 315 (1916).

[214] Dimroth, Scheurer: *Ann.*, **399**, 48 (1913).

[215] Tschirch, Farner: *Arch. Pharm.*, **237**, 35 (1899); *Schweiz. Apoth. Z.*, **60**, 609 (1922); Tschirch, Lüdy, Jr.: *Helv. Chim. Acta*, **6**, 994 (1923); D. R. P. 226,880 (Fowler) Friedl., **10**, 975.

[216] Lac-dye is difficult to obtain in commerce and its mineral content makes it difficult to work.


oxidation of the trimethyl derivative with KMnO4
Br2
CH3COOH
HNO3
Kermesic Acid
Nitrococcussic Acid
Br2
CH3COOH
Reduction
Zn + CH3COOH
Monobromococcin

but somewhat faster dyeings than cochineal, *e.g.*, on wool with a tin mordant beautiful scarlet and on aluminum mordant carmin red shades are produced. Stick-lac contains, according to Dimroth, 0.5-0.75 per cent of coloring matter, termed laccaic acid by R. E. Schmidt [217] who first investigated it. The preparation of completely ash-free pigment presents some difficulty. Ground stick-lac is extracted with water (Dimroth [218]), the solution acidified with acetic acid, filtered and concentrated. On addition of hydrochloric acid to the solution the pigment is slowly precipitated, and is recrystallized from formic acid in microscopic rhombohedra.

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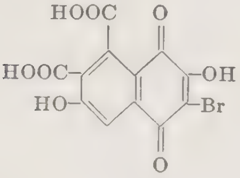
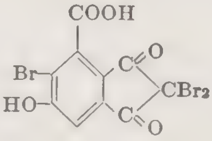
[217] R. E. Schmidt: *Ber.*, 20, 1285 (1887).
[218] Dimroth, Goldschmidt: *Ann.*, 399, 62 (1913).

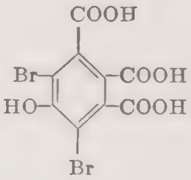
Schmidt suggested the empirical formula $C_{16}H_{12}O_8$, but Dimroth's researches suggest that $C_{20}H_{24}O_{10}$ is correct; the latter formula is based on comparative analyses of laccaic acid and the product $C_{20}H_{16}O_9$ obtained by reducing it with zinc dust and ammonia. The latter, on mild oxidation, passes into a pigment, $C_{20}H_{14}O_9$, which bears to the reduction product the relation of a quinone to a hydroquinone. Five hydrogen atoms in laccaic acid are replaceable by metal. Oxidation with hydrogen peroxide in alkaline solution in the presence of metal salts (cobalt or manganese) yields calaic acid, $C_{18}H_{14}O_{11}$ (yellow prisms), which contains three carboxyl groups, at least one carbonyl group, and is probably a naphthoquinone derivative. By the action of bromine on calaic acid in acetic acid, two substances are produced, one yellow and one colorless acid, of which only the first is regarded as possessing any significance in elucidating the structure of the pigment. This, $C_{12}H_5O_8Br$ (needles, m.p. 234-235°) is an analog of β-bromocarmin and was termed β-bromolaccain (I). In β-bromolaccain, four hydrogen atoms are still replaceable by metals and two hydrogen atoms may be acetylated, although anhydride formation takes place on acetylation.



(I)

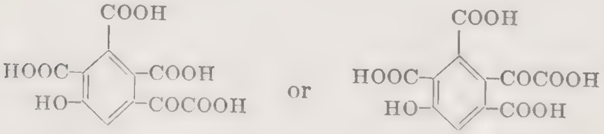


(II)

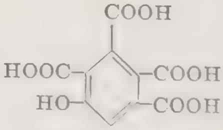


(III)

Further bromination of β-bromolaccain in aqueous solution leads to an indandione derivative, α-bromolaccain (II), crystallizing in colorless needles, which with hydrobromite passes into a dibromphenoltricarboxylic acid (III) (cubes, m.p. 257-258°). The structure is confirmed by the fact that the acid gives only a weak coloration with ferric chloride and contains no hydroxyl group *ortho* to a carboxyl group. β-Bromolaccain was also further degraded by hydrogen peroxide in acetic acid solution when a ketoacid (IV) was obtained, which was converted by warm concentrated sulfuric acid into a phenol tetracarboxylic acid (V):

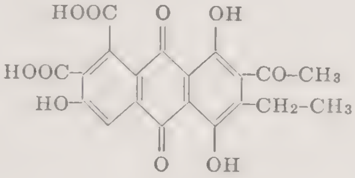


(IV) *Tablets*
(*m.p. 229.5-230°*)



(V) *Plates*
(*m.p. 212-214°*)

From this investigation which was interrupted at this point it may be concluded that lac-dye is a hydroxyanthraquinone carboxylic acid and that this formula may be suggested:



According to Tschirch[219] stick-lac contains a second yellow coloring matter, erythrolaccain, $C_{15}H_{10}O_6$ (red crystals), to which shellac owes its color. It yields a tetracetyl derivative and is probably therefore a tetrahydroxymethylanthraquinone.

Lanigerin. This pigment[220] occurs in the woolly aphis, *Eriosoma lanigerum* Hausmann, popularly known as American blight, and is obtained by extraction with ether (0.2 per cent of the weight of the insects). Lanigerin has a composition corresponding to $C_{17}H_{14}O_7$ (orange leaflets, m.p. 274-276°) and contains three active hydrogen atoms. It gives green fluorescent solutions in ether and acetic acid, and it is suggested that its structure is that of a polyhydroxyanthraquinone with a quinizarin grouping.

Santalin is present in sandal-wood from the *Leguminosae Pterocarpus santalinus* and *Pterocarpus indicus,* indigenous to the East Indies, Ceylon, Golconda and Timor. Caliatur (or carratur) wood is identical with sandal-wood. The wood comes into commerce in the form of hard, red blocks which in ground or rasped form may be used for dyeing wool and cotton. The pigment dyes directly, but mordanted wool is preferable when red shades are to be obtained. The dyeings are fast to acid but unstable to light and alkali; tin mordants present no marked advantages. Santalin appears to be present also in the wool of *Baphia nitida* from Sierra Leone.

The constitution of santalin is not yet completely established. Pelletier[221] was the first to examine santalin in 1814, and a process for preparing it has been evolved from many later researches,[222] particularly those of Perkin and Cain, whereby the pigment is extracted with alcohol, precipitated with barium hydroxide and purified through the barium salt. Besides santalin, a somewhat more readily soluble desoxysantalin has been isolated. Later, Dieterle and Stegemann,[223] employing another method of isolation, claimed to have separated two pigments (A and B). Santalin obtained by them and purified through the potassium salt forms a red microcrystalline powder which softens at 218° and carbonizes above 300°. Their analyses[224] agree either with $C_{15}H_{14}O_5$ (Cain and Simonsen) or with $C_{24}H_{22}O_8$ (O'Neill and Perkin), of which the first is confirmed by molecular weight determinations of a number of derivatives. Raudnitz, Navrátil and Benda[225] have obtained an oxonium salt of formula $C_{34}H_{29}O_{10}Cl$, from which they deduce that santalin is $C_{34}H_{28}O_{10}$ or, more particularly, $C_{30}H_{16}O_6(OCH_3)_4$. According to earlier results santalin contains only one methoxyl group but takes up two acetyl or two benzoyl residues directly, and two more acetyl groups are introduced by reductive acetylation. A quinone structure is thus indicated and the presence of an anthracene derivative may be deduced from the formation of that hydrocarbon on distillation with zinc dust.

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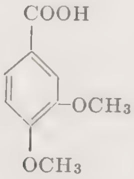
[219] Tschirch, Farner: *Arch. Pharm.,* **237,** 35 (1899); *Schweiz. Apoth. Z.,* **60,** 609 (1922); Tschirch. Lüdy, Jr.: *Helv. Chim. Acta,* **6,** 994 (1923).

[220] Blount: *J. Chem. Soc.,* **1936,** 1034.

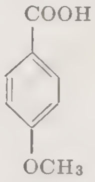
[221] Pelletier: *Ann.,* **6,** 28 (1833).

[222] Bolley: *Ann.,* 62, 150 (1847); Meier: *Arch. Pharm. (2),* **55,** 285 (1848); *(2),* **56,** 41 (1849); Weyermann, Häffeley: *Ann.,* **74,** 226 (1850); Preisser: *Berzelius Jahresber.,* **24,** 515 (1845); Weidel: *Z. Chem. (n. s.),* **6,** 83 (1870); Franchimont: *Ber.,* **12,** 14 (1879); A. G. Perkin: *J. Chem. Soc.,* 75, 443 (1899); v. Cochenhausen: *Angew. Chem.,* **17,** 874 (1904); Brooks: *Philippine J. Sci. (A),* **5,** 439 (1910); Cain, Simonsen: *J. Chem. Soc.,* **101,** 1061 (1912); Cain, Simonsen, Smith: *Ibid.,* **105,** 1339 (1914); O'Neill, A. G. Perkin: *J. Chem. Soc.,* **113,** 125 (1918).

Earlier workers had isolated from oxidation experiments veratric acid (I), resorcinol, and anisic acid (II); and oxidative degradation of a nitro-



(I)



(II)

santalin dimethyl ether gave, besides anisic acid, 4-nitro-2,3-dimethoxybenzoic acid, from which it may be concluded that the santalin employed contained both components A and B. Dieterle and Stegemann obtained, by oxidizing with hydrogen peroxide, a compound $C_{15}H_{10}O_7$ (yellowish-brown crystals, m.p. 125°), in which four hydrogen atoms have been replaced by two oxygen atoms. By more drastic degradation with nitric acid they obtained oxalic acid and styphnic acid (1,3-dihydroxy-2,4,6-trinitrobenzene) already obtained by Franchimont. On these results Dieterle and Stegemann suggested therefore the formula (III):

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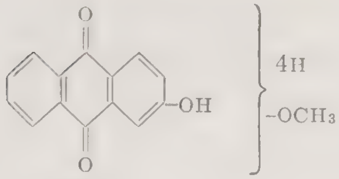
[223] Dieterle, Stegemann: *Arch. Pharm.*, **264**, 1 (1926).

[224] Brooks [*Philippine J. Sci. (A)*, **5**, 439 (1910)] has assigned the formula $Cu(C_{15}H_{13}O_3)_2$ to a copper salt.

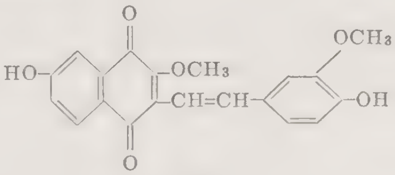
[225] Raudnitz, Navrátil, Benda: *Ber.*, **67**, 1036 (1934); cf. also Leonhardt, Buscke: *Ber.*, **67**, 1403 (1934), who claim to have isolated a similar substance pterosantalin (decom. 318°); see also Raudnitz: *Ber.*, **67**, 1603 (1934) and Leonhardt, Buscke: *Ber.*, **67**, 1888 (1934).



(III)

It must be pointed out however that an anthraquinone formula is not free from doubt when the structure of desoxysantalin and homopterocarpin (see below) are taken into consideration; moreover it can hardly be directly correlated with the empirical formula of Raudnitz.

For desoxysantalin[226] the formula $C_{20}H_{16}O_6$ has recently been suggested, this being supported by examination of its copper and sodium salts. The compound yields a diacetyl compound (salmon-colored crystals, m.p. 165-166°) and on reductive acetylation a tetracetyl compound (yellowish crystals, m.p. 184-185°). Of the six oxygen atoms present, two are in the form of hydroxyl groups, two as quinone groups, and a Zeisel determination shows that the last two are present as methoxyl groups. Ozonolysis yields vanillin and a second fission product, an acid of the formula $C_{12}H_{28}O_6$ (red needles, m.p. 112-113°) which is regarded as being a hydroxymethoxynaphthoquinone carboxylic acid. 2-Hydroxyphthalic acid is obtained on ozonization of the diacetate of desoxysantalin. The point of attack in these ozonolyses is a single double-linking, as is proved by catalytic hydrogenation. Formula (IV) is suggested on the basis of these observations.



(IV)

According to Anderson[227] the wood of *Baphia nitida* contains, in addition to desoxysantalin, a compound baphiin, of composition $C_{24}H_{20}O_8$, which melts at 200° and from which a number of derivatives were obtained, including baphnitone, $C_{26}H_{26}O_6$(?), which may be identical with homopterocarpin to be discussed below.

In addition to the pigments described above, three colorless substances[228] have been isolated from sandal-wood: pterocarpin, $C_{17}H_{14}O_5$

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[226] Engelhardt: "Über einen von Perkin als Desoxysantalin bezeichneten Farbstoff aus dem roten Sandelholz," Diss., Frankfurt, 1931.

[227] Anderson: *J. Chem. Soc.*, 30, 582 (1876).

[228] Cazeneuve, Hugounenq: *Compt. rend.*, 104, 1722 (1887); 107, 737 (1888); *Bull. soc. chim. (2)*, 48, 86 (1887); Brooks: *Philippine J. Sci. (A)*, 5, 439 (1910); Ryan, Fitzgerald: *Proc. Roy. Irish Acad. (B)*, 30, 106 (1913); Dieterle, Stegemann: *Arch. Pharm.*, 264, 1 (1926); Dieterle, Leonhardt: *Ibid.*, 267, 81 (1929); Leonhardt, Fay: *Ibid.*, 273, 53 (1935); Leonhardt, Oechler: *Ibid.*, 273, 447 (1935); Raudnitz, Perlmann: *Ber.*, 68, 1862 (1935).

(m.p. 165° or 156-157°; two interconvertible modifications have been described), which is levo-rotatory and contains a difficultly characterized carbonyl group, an ether oxygen atom, a methoxyl group and possibly a methylene oxide group; homopterocarpin, $C_{17}H_{16}O_4$ (m.p. 83-84°), containing two methoxyl groups, one carbonyl group and one oxygen atom bound as an ether, gives resorcinol dimethyl ether and methylanthracene on distilling with zinc dust; and santal, $C_{13}H_{10}O_5$ or $C_{12}H_5O_2(OH)_2(OCH_3)$, (yellowish needles, m.p. 218°).

These compounds are extracted from sandalwood with ether after triturating with lime and the residue from the ether extraction dissolved in chloroform. On adding alcohol, pterocarpin is precipitated while homopterocarpin remains in solution; 100 g of sandal-wood yields 0.4-0.6 g of homopterocarpin and 0.8-0.11 g of pterocarpin. Brooks, who found the two last-named compounds in narra-wood prior to the work of Dieterle and Leonhardt, ascribed somewhat different formulas.

Weidel[229] asserts that sandal-wood contains two further coloring materials which O'Neill and A. G. Perkins[230] believe they have found more recently in the wood of *Baphia nitida*. It is questionable whether these are identical with pigments A and B above.

Isosantalin. According to O'Neill and Perkin,[230] cam-wood, the product of a variety of *Baphia nitida,* contains a substance isomeric with santalin which is isolated in the same manner as santalin is isolated from sandal-wood. It has the composition $C_{24}H_{24}O_8$, forms a chocolate-brown powder darkening at 280° and decomposing at 290-300°, and gives bluish dyeings. Desoxyisosantalin, $C_{24}H_{22}O_7$ or $C_{24}H_{24}O_7$, corresponds to desoxysantalin, although these formulas require confirmation.

Durasantalin.[231] Dura red or sikhytan is a coloring matter used in Egypt and is obtained from *Andropogon sorghium,* var. *vulgaris.* It has been found also in the pods of the Manchurian kaoliang. It has been obtained as a pale-red powder to which the formula $C_{16}H_{12}O_5$ was provisionally given, although Okano[232] more recently favors $C_{48}H_{39}O_{15}$. Its violet-red alkaline solution becomes brown by atmospheric oxidation, and the pigment is broken down, on fusing with potash, into phloroglucinol and *p*-hydroxybenzoic acid. It is obtained by extracting the plant with acetone and fractionally precipitating the extract with benzene. Durasantalin dyes mordanted and unmordanted wool, but not mordanted cotton.

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[229] Weidel: *Z. Chem.,* **6,** 83 (1870).
[230] O'Neill, A. G. Perkin: *J. Chem. Soc.,* **113,** 125 (1918).
[231] A. G. Perkin: *J. Chem. Soc.,* **97,** 220 (1910).
[232] Okano, Ohara: *Chem. Zentr.,* **1936,** I, 94.

Narrin. This pigment,[233] which resembles santalin in its tinctorial properties, is obtained by alcohol extraction of narra-wood from species of *Pterocarpus* growing in the Philippine Islands. It forms a dark-red amorphous powder decomposing at 180° and gives a copper salt; fusion with alkali yields phloroglucinol and resorcinol.

Ventilagin [234] is one of a number of pigments found in the root-bark of *Ventilago madraspatana,* one of the *Rhamnaceae,* a shrub of climbing habit growing in the West Indies, Ceylon and Burma. The root-bark comes into commerce in the form of dark red or brown chips, shavings or fiber and is known under the names pitti, raktapita, pappili-chakka, suralpattai, lokandi and konwait. Ventilagin is a red-brown brittle resinous product of composition corresponding to $C_{15}H_{14}O_6$, which softens at 100° and melts at 110°. It dissolves in alkalies to purple-red solutions and on distillation with zinc dust yields α-methylanthracene. It is obtained by extracting the powdered root-bark, which contains 8-10 per cent of coloring matter, with carbon disulfide in the cold, and then separating accompanying impurities by a laborious precipitation with alcohol. Ventilagin dyes mordanted cotton, wool and silk, *e.g.,* an aluminum mordant is colored red.

PHENANTHRENE PIGMENTS

Thelephoric acid. This pigment [235] occurs in practically all *Thelephora* species which have been examined (*Thelephora palmata* Scop., *flabelliformis* Fr., *caryophyllea* Schaeff., *terrestris* Ehr., *coralloides* Fr., *crustacea* Schum., *intybacea* Pers., *laciniata* Pers.), *e.g.,* in grey *Basidiomycetes* found in heath-land and in pine forests in the autumn. It has been found in another fungus, *Hydnum ferrugineum* Fr. A preparation from *Thelephora palmata* (coriaceous millepore) by Kögl, which served for the determination of the constitution of thelephoric acid, had the formula $C_{20}H_{12}O_9$ and consisted of almost black prisms with a metallic luster, which were insoluble in all the common solvents except pyridine. The change of color of the wine-red solution in pyridine to corn-flower-blue on adding water is characteristic. The function of the nine oxygen atoms is revealed by the formation of an orange-yellow triacetyl derivative, a colorless pentaacetyl leucoderivative, and a heptamethylhexahydrothelephoric acid; the pigment thus contains three phenolic hydroxyl groups, two carboxylic groups and a quinonoid system. On distilling the triacetate with zinc dust an olefinic hydrocarbon, $C_{18}H_{14}$, was obtained, which was later shown to be 2′-phenanthryl-1-butadiene:

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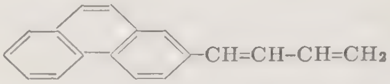
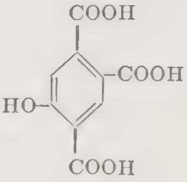
[233] Brooks: *Philippine J. Sci. (A),* **5,** 439 (1910).

[234] A. G. Perkin, Hummel: *J. Chem. Soc.,* **65,** 923 (1894).

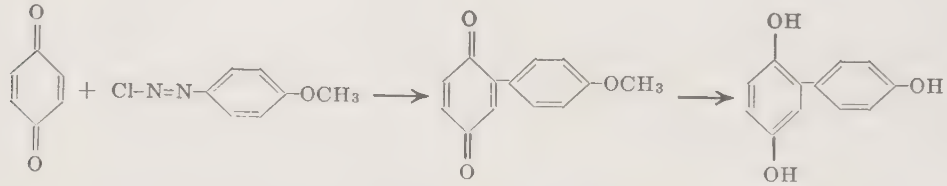
[235] Zopf: *Bot. Ztg.,* **47,** 69 (1889); Kögl, Erxleben, Jänecke: *Ann.,* **482,** 105 (1930). Occurrence of thelephoric acid in lichens: Asahina, Shibata, *Ber.,* **72,** 1531 (1939).



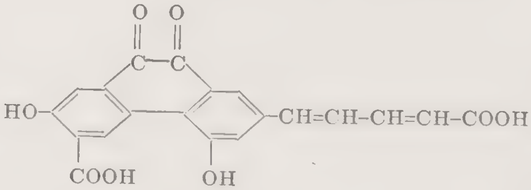
In accordance with this structure it was converted into phenanthrene-2-carboxylic acid on oxidizing with potassium permanganate. Thelephoric acid was degraded by hydrogen peroxide to hydroxytrimellitic acid (I), and the hydrogenated thelephoric acid yields not only (I) but also adipic acid. (I) was also obtained by oxidizing triacetylthelephoric acid with chromic acid, being accompanied by a compound $C_{16}H_{10}O_{11}$ (colorless needles, m.p. 290°) which was proved to be trihydroxydiphenyltetracarboxylic acid. From the latter 4,3′,6′-trihydroxydiphenyl, synthesized[236] as shown below, and thence *p*-hydroxybenzoic acid, were obtained:



(I)



All these observations find expression in the formula:



Thelephoric acid is extracted from the fungoid material by allowing it to swell in water and treating with pyridine. The yield is about 0.5 per cent of the dry weight. Thelephoric acid may be used as a vat-color in ammoniacal solution and dyes wool and cotton.

Tanshinone, $C_{18}H_{12}O_3$, m.p. 232-234°, occurring in the Chinese drug tan-shin, behaves as an *o*-quinone toward *o*-phenylene diamine. This quinone nature is confirmed by reductive acetylation; and as tanshinone gives 1-methylnaphthalene-5:6-dicarboxylic acid on oxidation, it is formulated[236a] as a phenanthraquinone, probably of the type:

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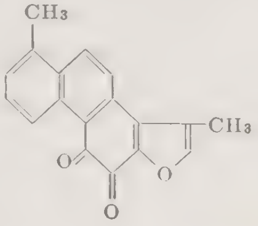
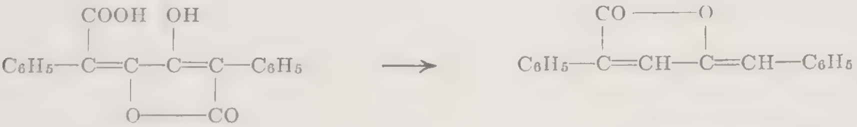
[236] U. S. Pat. 173,432 (I. G.).



Xylindein. Fallen and rotting branches of beech, oak or birch are frequently colored green by a fungus *Peziza aeruginosa.* The pigment, xylindein,[237] or wood-indigo, has the formula[238] $C_{34}H_{26}O_{11}$. A yellow tetraacetate and diacetylxylindein dimethyl ether (yellow needles, m.p. 294-295°) indicate the presence of four hydroxyl groups; two more oxygen atoms probably exist in a quinonoid system, and the remaining five oxygen atoms must be bound as oxides or lactones. Now, on treating xyleindein with caustic soda, it dissolves as a green sodium salt, taking up four equivalents of alkali. These four equivalents correspond to two hydroxyl and two carboxyl groups as the sodium salt undergoes double decomposition with two equivalents of silver nitrate, and the resulting silver salt gives a dimethyl ester of xylindeinic acid, $C_{36}H_{34}O_{13}$. Thus in the formation of the tetra-sodium salt, two phenolic hydroxyls and two carboxyl groups participate and these themselves arise by the fission of two lactone rings. The introduction of two new hydroxyl groups should permit the preparation of a hexaacetyl derivative of the ester, but it appears that two of the hydroxyl groups are enolic in origin and only a tetraacetyl compound, $C_{44}H_{42}O_{17}$, has been obtained. In confirmation of this postulate the dimethyl ester of xylindeinic acid gives a crystalline disemicarbazone, whereas reductive acetylation of the ester gives a hexaacetate which no longer forms any semicarbazone. The behavior on catalytic reduction is also in agreement with this view and the whole character recalls the conversion of vulpinic into cornicularic acid[239]:



$$C_6H_5-\overset{COOH}{\overset{|}{C}}=\underset{\underset{O}{|}}{C}-\overset{OH}{\overset{|}{C}}=\underset{\underset{CO}{|}}{C}-C_6H_5 \longrightarrow C_6H_5-\overset{CO}{\overset{|}{C}}=CH-\overset{O}{\overset{|}{C}}=CH-C_6H_5$$

Vulpinic acid *Cornicularic acid*

[236a] Nakao, Fukushima, *J. Pharm. Soc. Japan*, **54,** 844 (1940); Wessely, Wang, *Ber.*, **73,** 19 (1940).

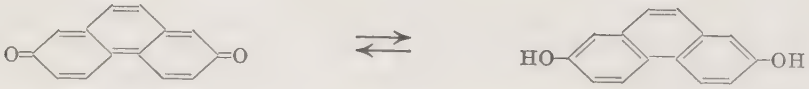
[237] Rommier: *Compt. rend.*, **66,** 108 (1868); Liebermann: *Ber.*, **7,** 1102 (1874).

[238] Kögl, v. Taeuffenbach: *Ann.*, **445,** 170 (1925); (see here also the older literature); Kögl, Erxleben: *Ann.*, **484,** 65 (1930).

[239] Spiegel: Ann., **219,** 3 (1883) and p. 37.

The quinone nature may be regarded as established, although the instability toward permanganate and the resistance to hydrogenation suggest that a more complicated system exists in which the double-bonds assume a polyene nature by being continued through a number of nuclei. The uptake of two hydrogen atoms as in a vat process is then associated with establishing a benzenoid system. On treating with alcoholic potash, acetic and *n*-butyric acids are formed, together with a brown precipitate, the acetyl derivative of which forms a yellow compound, $C_{13}H_8O_{10}$ or $C_{13}H_{10}O_{10}$.

On distillation of the dimethyl ester of hexaacetyltetrahydroleucoxylindeinic acid with zinc dust, phenanthrene is obtained. It is possible therefore that xylindein is a diphenanthryl derivative, a view which may plausibly be correlated with the unusual behavior of the quinone mentioned above:



Xylindein is obtained by extracting green rotting beech-wood with warm phenol and adding water to the extract.

Strobinin [240] is obtained from the chloroform or ether extract of the white pine chermes, *Adelges* (*Pineus*) *strobi* Börner. It has the formula $C_{30}H_{24}O_8$, forms dark-red brown prisms (m.p. 236-237°), contains three active hydrogen atoms and exhibits color reactions, suggesting a hydroxyphenanthraquinone structure.

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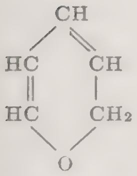
[240] Blount: *J. Chem. Soc.*, **1936**, 1034.

Chapter 4

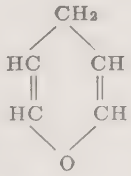
Heterocyclic Compounds

COMPOUNDS CONTAINING HETEROCYCLIC OXYGEN ATOMS

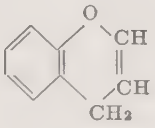
This section comprises a number of lichen pigments containing a five-membered lactone ring and also yellow, red and blue flower-coloring substances, the constitution of which has been completely elucidated. There are included also a number of compounds which probably but not certainly, as far as is yet known, belong to this group; and finally red and blue wood- and xanthone pigments are discussed. The flower pigments are all derived from γ- or 1,4-pyrane[1] (I):



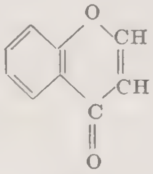
(A)



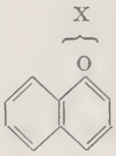
(I)



(II)

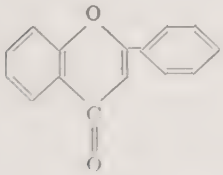


(III)

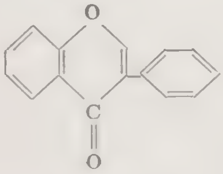


(IV)

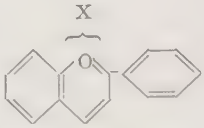
and more particularly from α-β-benzo-γ-pyrane (II). They may be further subdivided into yellow pigments containing a carbonyl group of the type of chromone (III), and on the other hand red and blue coloring matters, which are benzopyrilium salts with the basic structure (IV). With the exception of daphnetin, all the naturally occurring pigments of this group contain at least one additional benzene ring and possess structures of the following types:



Flavone



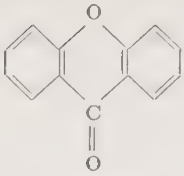
Isoflavone



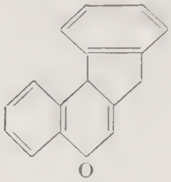
Flavylium salts

---

[1] Only one compound possessing tinctorial properties and derived from α- or 1,2-pyrene (A) has been found in nature.



Xanthone

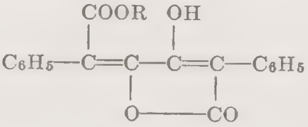


(V)

Red and blue wood colors are derived from a system in which indane and chromane nuclei are fused together, *i.e.*, from the hydrindenochromane (V).

PIGMENTS CONTAINING A FIVE-MEMBERED RING

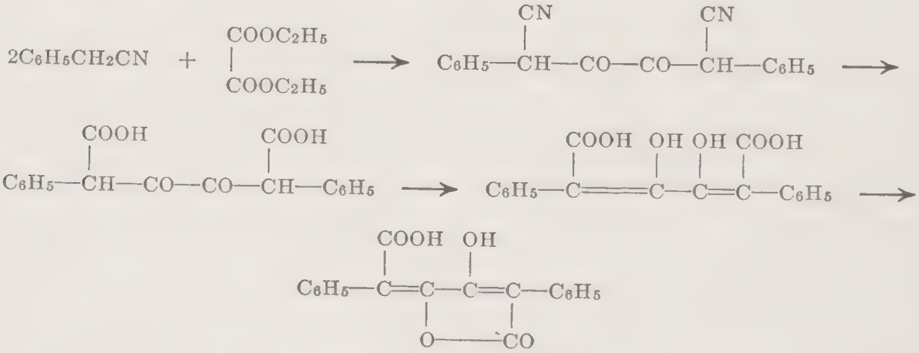
Vulpinic acid. This pigment[2] is found in lichens such as *Letharia vulpina, Cetraria tubulosa,* in *Cypheliaceae, Parmeliaceae, Usneaceae,* etc. It has the formula $C_{19}H_{14}O_5$ (yellow leaflets, m.p. 148°), and is the methyl ester of pulvinic acid, $C_{18}H_{12}O_5$, a lactonic carboxylic acid of the structure:


COOR
OH
C₆H₅—C=C—C=C—C₆H₅
O
CO

R=H Pulvinic acid

R=CH_3 Vulpinic acid

The constitution of vulpinic acid has been completely proved by the following synthesis of pulvinic acid[3]:


2C₆H₅CH₂CN
COOC₂H₅
COOC₂H₅
CN
CN
C₆H₅—CH—CO—CO—CH—C₆H₅
COOH
COOH
C₆H₅—CH—CO—CO—CH—C₆H₅
COOH OH OH COOH
C₆H₅—C=C—C=C—C₆H₅
COOH OH
C₆H₅—C=C—C=C—C₆H₅
O
CO

the diketo acid first obtained passing into the dienolic form. The acid is isolated by extracting the lichens with lime and precipitating with acid.

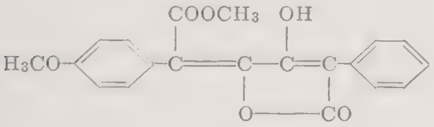
Pinastrinic acid (chrysocetraric acid). This acid,[4] which occurs in *Cetraria pinastris* and more abundantly in *Cetraria juniperina,* together with vulpinic and usnic acid, has the formula $C_{20}H_{16}O_6$ (yellow needles, m.p. 203-204°) and differs from vulpinic acid only by the group CH_2O. Koller and Pfeiffer disproved previous assertions by demonstrating the presence of two methoxyl groups. Moreover, as oxidation with potassium permanganate gave anisic and benzoic acids and hydrolysis with baryta yielded oxalic, phenylacetic, and *p*-methoxyphenylacetic acids, pinastrinic acid probably possesses the following structure:

---

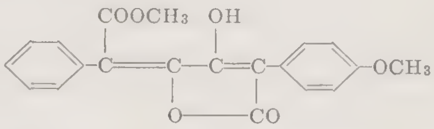
[2] Older literature: V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," **II**, 2, p. 268; especially Spiegel: *Ber.*, **13**, 1629, 2219 (1880); **14**, 1686 (1881); **15**, 1546 (1882); *Ann.*, **219**, 1 (1883).

[3] Volhard: *Ann.*, **282**, 1 (1894); reduction: Asano, Kameda: *Ber.*, **68**, 1565 (1935).

[4] Koller, Pfeiffer: *Monatsh.*, **62**, 160 (1933); early literature: Hesse: *Ann.*, **284**, 157 (1895); *Ber.*, **30**, 357 (1897); *J. prakt. Chem. (2)*, **57**, 232 (1898); Zopf: *Ann.*, **284**, 107 (1895).

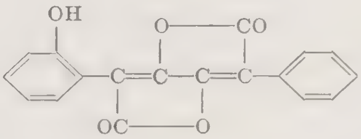


or



and this was confirmed by synthesis[5] from ethyl oxalate, *p*-methoxybenzyl cyanide and benzyl cyanide. The acid is isolated by extracting the lichens with ether and separating it from accompanying usnic acid by crystallization. Epanorin,[6] stictaurin,[7] and coniocylic acid[8] possibly belong to the pulvinic acid group.

Calycin,[9] occurring in the *Calyciaceae* and *Lepraria* species, has a composition corresponding to $C_{18}H_{10}O_5$ and forms orange-red needles (m.p. 244-245°). It is broken down by potassium hydroxide into oxalic, phenylacetic, and *o*-hydroxyphenylacetic acids and is *o*-hydroxypulvinic anhydride:



It has been synthesized from *o*-methoxybenzylcyanide, ethyl oxalate and benzylcyanide. Calycin is best isolated from the thalli of *Stictaurea* Ach.

Usnic acid. This acid[10] is one of the most widely distributed of the lichen coloring matters and has been found in about 70 *Usnea, Pamelia* and *Lecanora* species. It forms yellow crystals (m.p. 202-204°), and has the formula $C_{18}H_{16}O_7$. Earlier work has demonstrated that usnic acid contains an acetoacetic acid residue, and accordingly undergoes a ketonic hydrolysis on being heated with aqueous solvents with conversion into decarbousnic acid, $C_{17}H_{18}O_6$, which no longer contains a carboxylic group.

---

[5] Koller, Klein: *Monatsh.*, **63,** 213 (1933); reduction: Asano, Kameda: *Ber.*, **68,** 1565 (1935).

[6] Zopf: *Ann.*, **313,** 331 (1900).

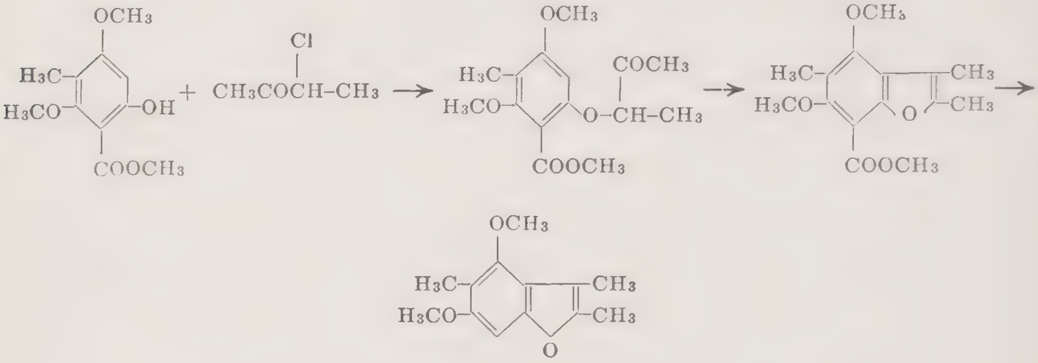
[7] Salkowski: *Ann.*, **319,** 391 (1902); Kappen: *Z. Krystallogr.*, **37,** 151 (1903); Zopf: *Ann.*, **306,** 283 (1899); **317,** 123 (1901); **338,** 35 (1905); Hesse: *J. prakt. Chem. (2)*, **62,** 333 (1900).

[8] Brieger: "Flechtenstoffe in Klein Handbuch der Pflanzenanalyse," **III,** 2, p. 413; *Ibid.*, Thies: "Systematische Verbreitung und Vorkommen der Flechtenstoffe," p. 429.

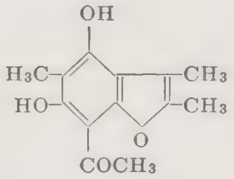
[9] Hesse: *Ber.*, 13, 1816 (1880); *J. prakt. Chem. (2)*, **62,** 321 (1900); Zopf: *Ann.*, **284,** 107 (1895); **346,** 82 (1906); Asano, Kameda: *Ber.*, 68, 1562 (1935).

[10] Schöpf, Heuck: *Ann.*, **459,** 233 (1927); a review of the older literature is given here.

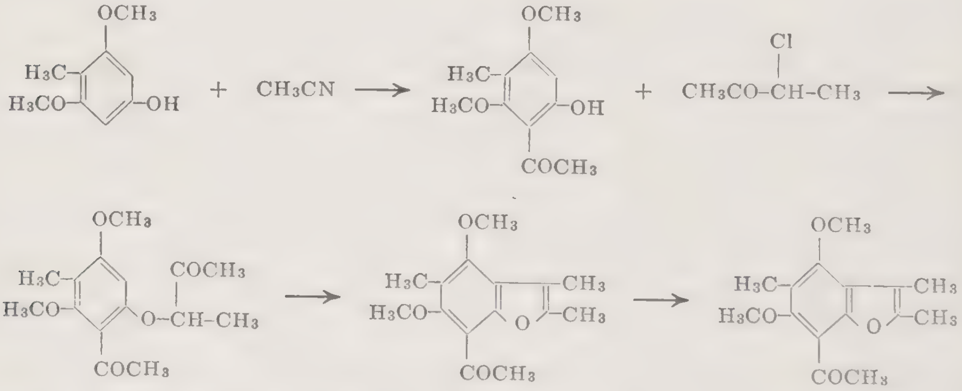
The elimination of the acetoacetic acid group by alkali treatment corresponds to an acid hydrolysis. The product, usnetic acid, $C_{14}H_{14}O_6$, may be decarboxylated to the phenol $C_{13}H_{14}O_4$, usnetol, and this on losing an acetyl group passes into usneol, $C_{11}H_{12}O_3$. Usneol is known to be 4,6-dihydroxy-2,3,5-trimethylcumarone from the synthesis[11] of its dimethyl ether:


OCH₃
H₃C
H₃CO
OH
COOCH₃
+
Cl
CH₃COCH-CH₃
OCH₃
H₃C
H₃CO
COCH₃
O-CH-CH₃
COOCH₃
OCH₃
H₃C
H₃CO
CH₃
CH₃
O
COOCH₃
OCH₃
H₃C
H₃CO
CH₃
CH₃
O

The results of the stepwise degradation of usnetol by ozone and alkali to C-methylphloroglucinol necessitate the following formula for usnetol:


OH
H₃C
HO
CH₃
CH₃
O
COCH₃

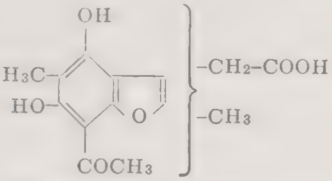
This has been confirmed by the synthesis of its dimethyl ether in the following manner:


OCH₃
H₃C
H₃CO
OH
+
CH₃CN
OCH₃
H₃C
H₃CO
OH
COCH₃
+
Cl
CH₃CO-CH-CH₃
OCH₃
H₃C
H₃CO
COCH₃
O-CH-CH₃
COCH₃
OCH₃
H₃C
H₃CO
CH₃
CH₃
O
COCH₃
OCH₃
H₃C
H₃CO
CH₃
CH₃
O
COCH₃

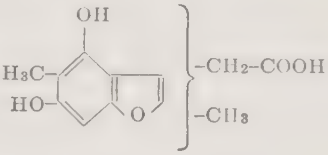
It may be noted that these synthetic results of Curd and Robertson led to a revision of the formulas of Schöpf and Heuck. The English workers succeeded also in showing that usnetic acid may lose the elements of acetic acid and simultaneously take up water to give pyrousnic acid, $C_{12}H_{12}O_5$, in agreement with the structures:

---

[11] Curd, Robertson: *J. Chem. Soc.*, **1933**, **437**, **715**, **1173**.

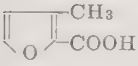

OH
H3C–
HO–
O
COCH3
–CH2–COOH
–CH3

Usnetic acid

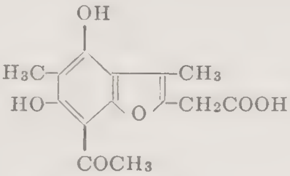

OH
H3C–
HO–
O
–CH2–COOH
–CH3

Pyrousnic acid

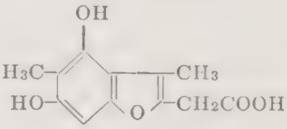
The orientation of the methyl and acetic acid side-chains was established when it was shown that the phloroglucinol nucleus could be oxidized away to give the acid (I):



(I)

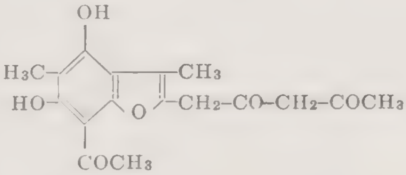

OH
H3C–
HO–
O
CH3
CH2COOH
COCH3

Usnetic acid (II)


OH
H3C–
HO–
O
CH3
CH2COOH

Pyrousnic acid (III)

so that usnetic and pyrousnic acid have structures (II) and (III) respectively—a conclusion[12] which has been justified by the synthesis of the dimethyl ether of the latter.[13] Decarbousnic acid, which it will be remembered passes into usnetic acid by loss of an acetyl group, must therefore be[14]:


OH
H3C–
HO–
O
CH3
CH2–CO–CH2–COCH3
COCH3

but the structure of usnic acid itself is still a debatable problem.[13-16] It was at one time thought that the acidity of usnic acid was due to a lactone ring, but for several reasons this view was abandoned by Curd and Robertson and later by other workers. One cogent reason was that the optical activity of usnic acid was not affected by solution in alkali and reacidification. Of the possible formulas for usnic acid which, to repeat, must be derived formally by addition of CO_2H to decarbousnic acid, followed by cyclization, formula (A) seems to accord best with the known facts[16a]:

---

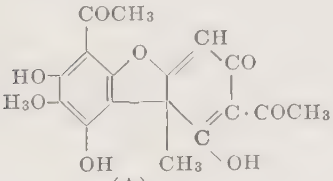
[12] Asahina, Yanagita: *Ber.*, **69**, 1646 (1936); **70**, 66 (1937); Asahina, Mayeda, Yanagita: *Chem. Zentr.*, 1937, II, 4049; Curd, Robertson: *J. Chem. Soc.*, **1937**, 894.

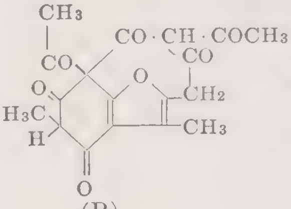
[13] Birch, Robertson: *J. Chem. Soc.*, **1938**, 306.

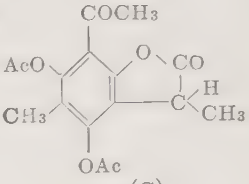
[14] Asahina, Yanagita: *Ber.*, **69**, 1646 (1936); **72**, 1140 (1939); Curd, Robertson: *J. Chem. Soc.*, **1937**, 894.

[15] Asahina, Yanagita, Mayeda: *Ber.*, **70**, 2462 (1937); Asahina, Yanagita: *Ibid.*, **71**, 2260 (1938); Yanagita: *Ibid.*, **71**, 2269 (1938).

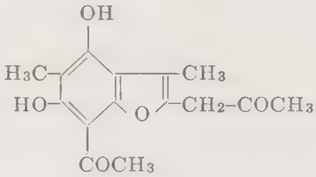
[16] Ross, Schöpf: *Naturwiss.*, **26**, 772 (1938).


(A)


(B)


(C)

In particular, the possible, though sterochemically unlikely alternative (B) seems to be effectively eliminated by the isolation of the diacetate of the coumaranone (C) on ozonizing usnic acid diacetate; (B) would give at most a monoacetate. Formula (A) is also in good accord with the formation of an oxime anhydride and an anhydro-bisphenylhydrazone by usnic acid. Such derivatives could be obtained from lactone structures, but it is more reasonable that they contain isoxazole and pyrazole systems respectively. Here again formula (B) seems to be eliminated, for the formation of these derivatives does not affect the asymmetric center; the anhydro-bisphenylhydrazone, for example, is known in *d*- and rac.-forms. Isodecarbousnic acid (m.p. 197°), discovered by Widmann,[17] is a degradation product[18] of the structure:



Usnic acid is isolated[19] from lichens, *e.g.*, *Usnea barbata* by extracting with ether or benzene. It appears likely that placodolic acid[20] belongs to the usnic acid series.

Citrinin[21] is produced on growing *Penicillium citrinum* on a glucose medium. It is an optically active monobasic acid, $C_{13}H_{14}O_5$, $[\alpha]_{\text{Hg green}} = -41.7°$ (alcohol), crystallizing in golden-yellow prismatic needles (m.p. 166-170°). It contains, in addition to the carboxyl group, one hydroxyl but no methoxyl or ketone groups. On hydrolysis with dilute sulfuric acid, a compound (I) is obtained containing two phenolic and one alcoholic hydroxyl group and one asymmetric carbon atom. When citrinin is fused with potash the products are carbon dioxide, formic acid and the methylethylresorcinol (II).

[16a] Foster, Robertson, Healy, *J. Chem. Soc.*, **1939**, 1594.

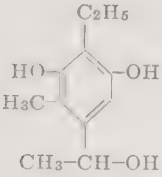
[17] Widmann: *Ann.*, **310**, 233 (1900).

[18] Curd, Robertson: *J. Chem. Soc.*, 1937, 894.

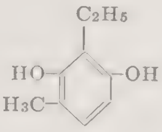
[19] Schöpf, Heuck: *Ann.*, **459**, 233 (1927).

[20] Zopf: *Ann.*, **297**, 285 (1897); **346**, 82 (1906); **340**, 295 (1905); Kappen: *Z. Krystallogr.*, **37**, 151 (1903).

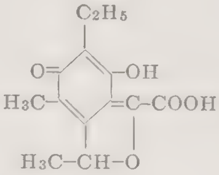
[21] Hetherington, Raistrick: *Philos. Trans. Roy. Soc. London (B)*, **220**, 209, 269 (1931); Coyne, Raistrick, Robinson: *Ibid.*, **220**, 297 (1931).



(I)



(II)

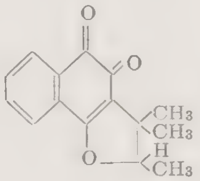


(III)

These observations thus find agreement in the structure (III). Citrinin is best isolated after infecting the glucose medium with the spores of the fungus and maintaining the culture at 28°. Thirty liters of culture solution contain 45-60 grams of citrinin.

Dunnione, $C_{15}H_{14}O_3$ (orange-yellow needles, m.p. 98-99°), is obtained from *Steptocarpus Dunnii* Mast.[21a]

To judge from its absorption spectrum, color reactions, reductive acetylation, and reaction with *o*-phenylene diamine, dunnione is an *o*-quinone and indeed a β-naphthoquinone. It closely resembles lapachol and similar α-naphthoquinones. The third oxygen atom is probably present in a chroman or coumaran ring, since the behavior of dunnione in alkalies and acids parallels closely that of α- and β-lapachones. Both β-lapachone and dunnione are thus formulated as β-naphthoquinones, and both isomerize in contact with strong acid, the latter to α-dunnione. Other isomerides of dunnione are also known. Of the possible formulations, the results of mild oxidation agree best with formula (A):



(A)

This formula is supported by the isolation of methylisopropyl ketone among the oxidation products.

Pigments Containing a Six-membered Ring

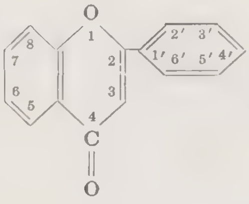
Flavone and Isoflavone Pigments[22]

The flavones comprise a very important class of plant pigments which are widely distributed in the vegetable kingdom especially as glycosides, but frequently in the free state or associated with tannin. Luteolin, known as weld or woad, the pigment used in Europe in ancient times, belongs to this group.

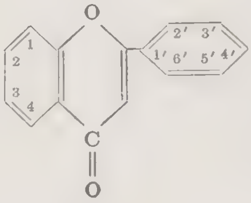
[21a] Price, Robinson, *Nature*, 142, 147 (1938); *J. Chem. Soc.*, 1939, 1522; 1940, 1493.

[22] Compilative account of the isolation of pigments, especially those of flavone character: *G. chimici*, 28, 299 (1934).

As mentioned above the natural pigments belong to the phenylflavone series. It must be understood that in the following account the carbon atoms are numbered according to scheme (I) whereas scheme (II) was often employed in the earlier literature:

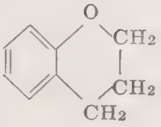

O
1
2
3
4
5
6
7
8
C
O
1′
2′
3′
4′
5′
6′

(I)

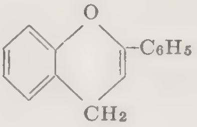

O
1
2
3
4
C
O
1′
2′
3′
4′
5′
6′

(II)

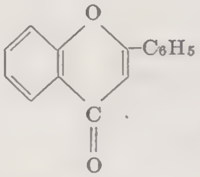
The following names distinguishing individual structures are noteworthy:


O
CH2
CH2
CH2

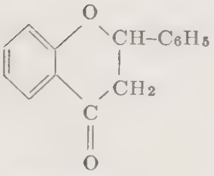
Flavane



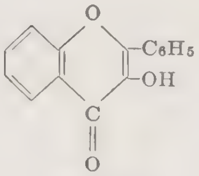
Flavene



Flavone

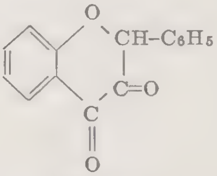

O
CH-C6H5
CH2
C
O

Flavanone



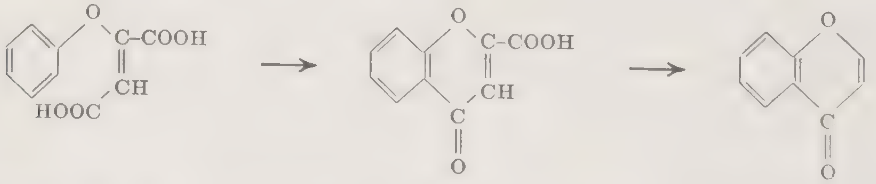
Flavonol
(enol form)

or


O
CH-C6H5
C=O
C
O

Flavonol
(keto form)

Chromone itself has been prepared by Ruhemann [23] by allowing phenoxyfumaric acid to stand with concentrated sulfuric acid and then distilling the chromone carboxylic acid so formed:


O
C-COOH
CH
HOOC
O
C-COOH
CH
C
O
O
C
O

In general, as was discovered by v. Kostanecki,[24] who made the study of natural flavone pigments derived from chromone his life's work, compounds of constitution

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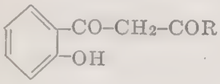
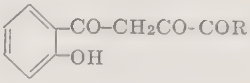
[23] Ruhemann and Stapleton: *J. Chem. Soc.*, **77**, 1179 (1900).

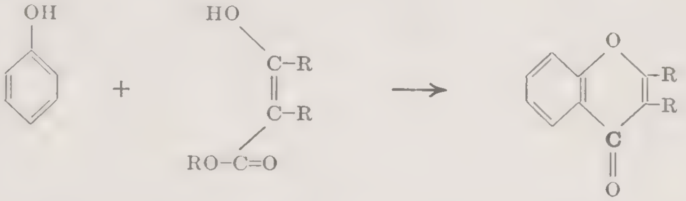
[24] v. Kostanecki: Conference 2, May 1903; *Bull. soc. chim. (3)*, **29**, XXVII (1903); v. Kostanecki's work is reviewed in his biography by Tambor: *Ber.*, **45**, 1701 (1913).



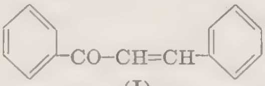
or



readily lend themselves to the synthesis of chromone derivatives. Finally, chromone derivatives are obtainable from phenol and alkylated acylacetic esters[25] in the following manner:

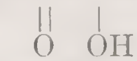


Chromone itself is colorless, but visible color appears when the pyrone ring carries a benzene ring, and hydroxyl groups are simultaneously introduced. The chromophoric group[26] in these flavone derivatives is



(I)
(parent compound)

—CO—C=C—. Its chromophoric power alone is small, but the orientation of the hydroxyl groups has a regular effect in the sense that hydroxyls in 5- and 7- positions exert only a minor action, whilst those in 3′- and 4′- positions produce a deep yellow shade. A hydroxyl in the 3- position produces only a pale yellow color, but it enhances the effect of a hydroxyl in positions 3′- and 4′- so that an orange compound is obtained.

Finally, in the hydroxy-flavones it is the grouping  $-\underset{\underset{\displaystyle O}{\|}}{C}-\underset{\underset{\displaystyle OH}{|}}{C}=$ which in the sense of Werner's postulates must account for their ability to form lakes with mordants; this property is shared by those compounds when the relative positions of the CO- and OH- groups are also 1,8. It has been observed that a hydroxyl group in position 5 is resistant to methylation.

Syntheses: (1) By the condensation of hydroxyacetophenone[27] with benzaldehyde or by condensation of further hydroxylated derivatives of these compounds [28]:

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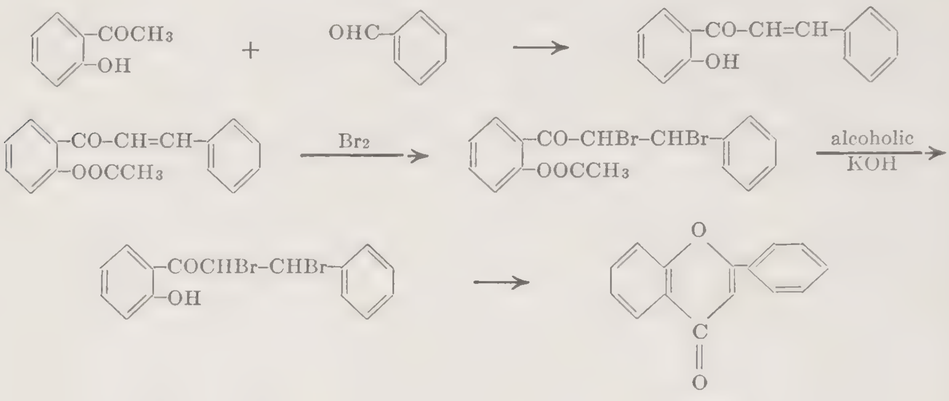
[25] Petschek, Simonis: *Ber.*, **46**, 2014 (1913).

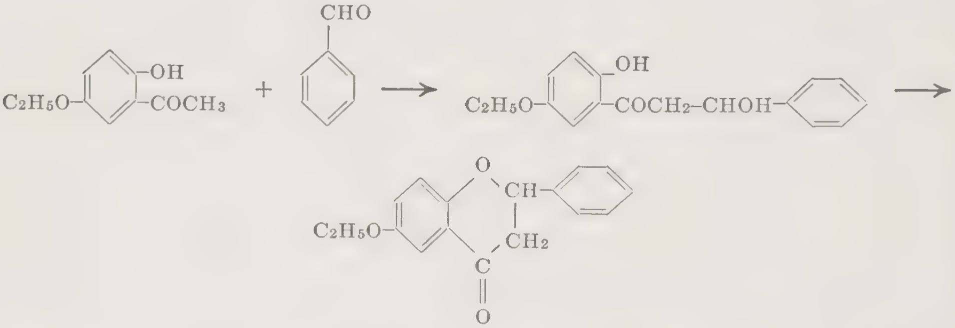
[26] The same group is encountered in the closely related chalkones (I) which are red-yellow.

[27] Emilewicz, v. Kostanecki: *Ber.*, 31, 696 (1898); cf. the use of the ω-chloro compound of hydroxyacetophenone: Simonis: *Angew. Chem.*, **39**, 1461 (1926).


$-COCH_3$
$-OH$
+
$OHC-$
$-CO-CH=CH-$
$-OH$
$-CO-CH=CH-$
$-OOCCH_3$
Br_2
$-CO-CHBr-CHBr-$
$-OOCCH_3$
alcoholic
KOH
$-COCHBr-CHBr-$
$-OH$
O
C
O

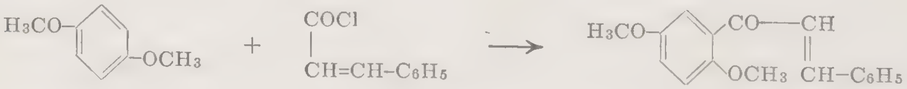
Flavone

If quinacetophenones are used (*i.e.*, hydroxyl groups in the 1,4 positions) then flavanones are obtained:


CHO
$-OH$
C_2H_5O-
$-COCH_3$
+
OH
C_2H_5O-
$-COCH_2-CHOH-$
O
CH
C_2H_5O-
CH_2
C
O

The dehydrogenation [29] of flavanone to flavone is effected by phosphorus pentachloride or selenium dioxide.

By the interaction of phenyl alkyl ethers, in which the *p*-position is preferably occupied, with cinnamic acid chloride chalkones [30] are formed:


H_3CO-
$-OCH_3$
+
COCl
$CH=CH-C_6H_5$
H_3CO-
CO
CH
OCH_3
$CH-C_6H_5$

and these on dealkylation undergo cyclization,[31] usually to flavanones but in some circumstances to flavones.

---

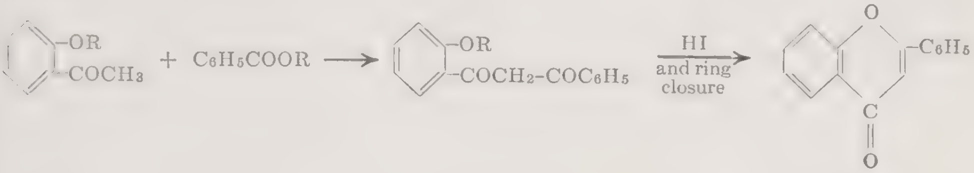
[28] On the formation of benzocumaranones, see Kesselkaul, v. Kostanecki: *Ber.*, **29**, 1886 (1896); v. Auwers, Pohl: *Ann.*, **405**, 293 (1914); v. Auwers: *Ann.*, **421**, 1 (1920); v. Auwers, Anschütz: *Ber.*, **54**, 1543 (1921). Improved method by heating above the melting point: Hutchins, Wheeler: *J. Chem. Soc.*, **1939**, 91.

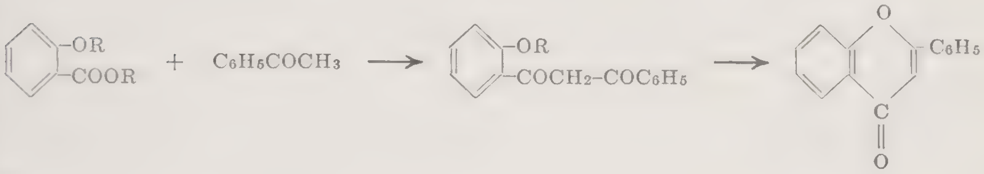
[29] Löwenbein: *Ber.*, **57**, 1515 (1924); Mahal, Rai, Venkataraman: *J. Chem. Soc.*, **1935**, 866; Virkar, Wheeler: *Chem. Zentr.*, **1939**, I, 664; Ullal, Wheeler: *Chem. Zentr.*, **1939**, I, 3375.

[30] Simonis, Lear: *Ber.*, **59**, 2908 (1926).

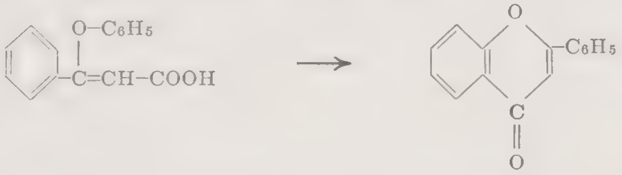
[31] Simonis, Danischewski: *Ber.*, **59**, 2914 (1926).

(2) By condensing any alkylated *o*-hydroxyacetophenone with an aromatic ester or an acetophenone with a salicyclic ester in presence of sodium[32]:

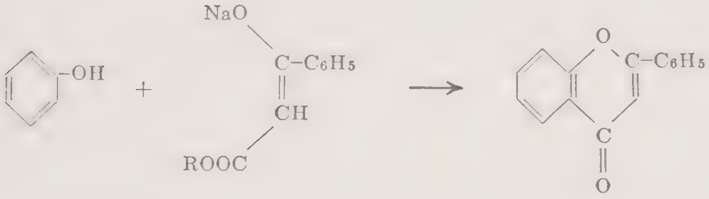

-OR
-COCH3
+ C6H5COOR
-OR
-COCH2-COC6H5
HI
and ring closure
O
-C6H5
C
O


-OR
-COOR
+ C6H5COCH3
-OR
-COCH2-COC6H5
O
C6H5
C
O

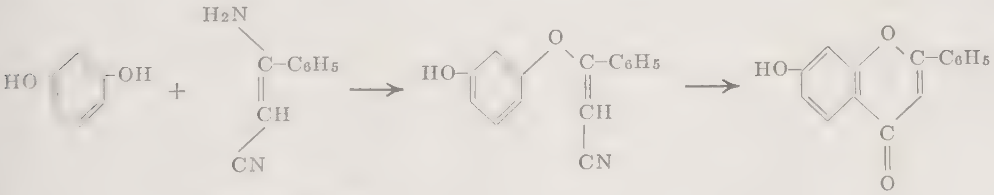
(3) By rearrangement of β-aryloxycinnamic acids[33] obtained by adding sodium phenoxide to phenylpropiolic acids:


O-C6H5
-C=CH-COOH
O
-C6H5
C
O

(4) By the action of phenols on sodio-benzoylacetic esters[34]:


-OH
+
NaO
C-C6H5
CH
ROOC
O
C-C6H5
C
O

The condensation of benzimidoacetonitrile with resorcinol[35] is a modification of this reaction:


HO
-OH
+
H2N
C-C6H5
CH
CN
HO-
O
C-C6H5
CH
CN
HO-
O
C-C6H5
C
O

(written in the tautomeric form)

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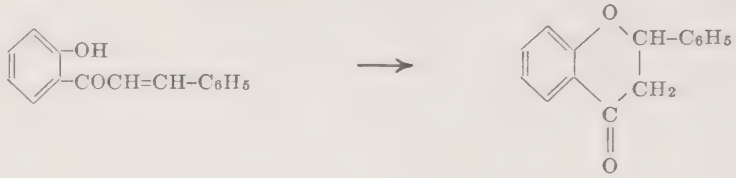
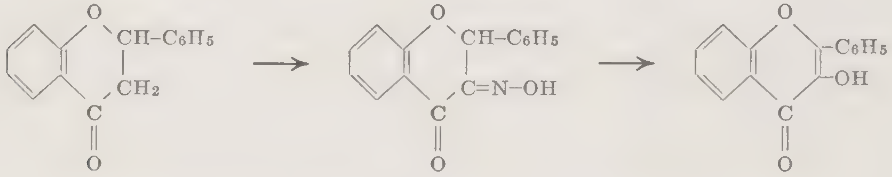
[32] v. Kostanecki, Tambor: *Ber.*, 33, 330 (1900).
[33] Ruhemann: *Ber.*, 46, 2188 (1913).
[34] Simonis, Remmert: *Ber.*, 47, 2229 (1914); cf. Ghosh: *J. Chem. Soc.*, 109, 105 (1916).
[35] v. Meyer: *J. prakt. Chem.* (2), 67, 342 (1903).

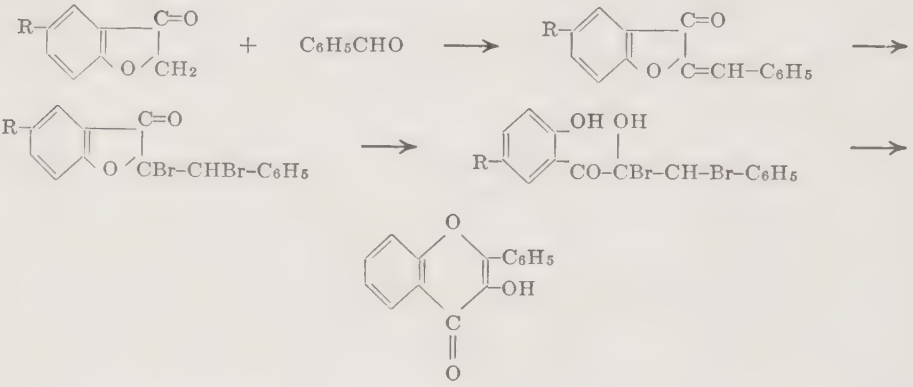
Turning to the formation of flavanones, it may be remarked that flavanone itself is produced by the rearrangement of ω-benzal-*o*-hydroxyacetophenone in boiling alcoholic hydrochloric acid[36]:


OH
COCH=CH–C6H5
O
CH–C6H5
CH2
C
O

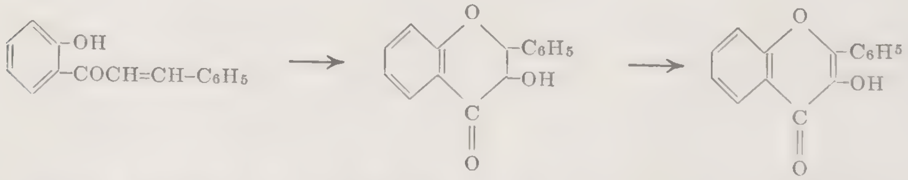
Flavanol[37] is obtainable from flavanone by treating with amyl nitrite and hydrochloric acid in alcoholic solution and then boiling the isonitroso compound so formed:


O
CH–C6H5
CH2
C
O
C=N–OH
C6H5
OH

Flavonols[38] are also obtained by ring fission of cumaranones:


R
C=O
O
CH2
+
C6H5CHO
C=CH–C6H5
CBr–CHBr–C6H5
OH OH
CO–CBr–CH–Br–C6H5
C6H5
OH
C
O

Somewhat similar is the conversion of *o*-hydroxychalkones[39] into flavonols by hydrogen peroxide in alkaline solution:


OH
COCH=CH–C6H5
O
C6H5
OH
C
O

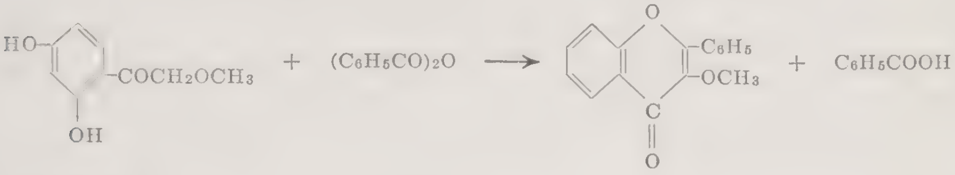
[36] v. Kostanecki, Szabranski: *Ber.*, **37**, 2634 (1904).

[37] v. Kostanecki, Lampe: *Ber.*, **37**, 773 (1904); cf. Algar, Flynn: *Proc. Roy. Irish Acad.* (*B*), 42, 1 (1934).

[38] Auwers, Müller: *Ber.*, **41**, 4233 (1908).

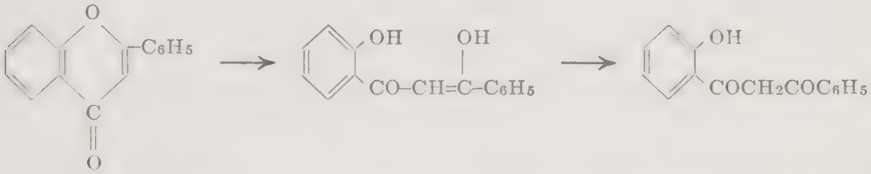
[39] Oyamada: *Bull. chem. soc. Japan*, **10**, 182 (1935); Murakami, Irie: *Chem. Zentr.*, 1935, II, 3653.

Finally flavonol derivatives are obtained by the interaction of, for example, ω-methoxyresacetophenone with benzoic anhydride [40]:

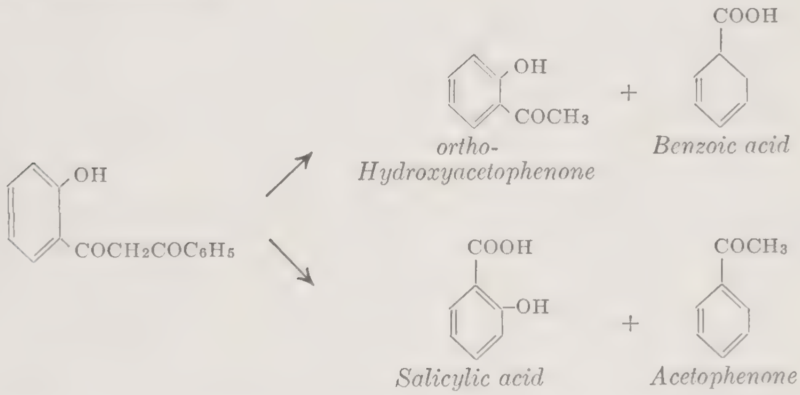

HO
COCH₂OCH₃
OH
+
(C₆H₅CO)₂O
O
C₆H₅
OCH₃
C
O
+
C₆H₅COOH

Degradation:

Flavone and its derivatives undergo a remarkable ring fission on treating with potassium hydroxide; from flavone a benzoyl-*o*-hydroxyacetophenone is first obtained; presumably by way of an enolic form:


O
C₆H₅
C
O
OH
OH
CO–CH=C–C₆H₅
OH
COCH₂COC₆H₅

and this is further degraded by "acid" and "ketone" hydrolysis so that four fission products arise:


OH
COCH₂COC₆H₅
OH
COCH₃
ortho-Hydroxyacetophenone
+
COOH
Benzoic acid
COOH
OH
Salicylic acid
+
COCH₃
Acetophenone

By such synthetic and degradative methods the constitution of all flavone derivatives may be demonstrated.

Shibata and Kimotsuki [41] were the first to study spectrographic relationships in the flavone series. It was shown that two absorption bands are present in the ultraviolet region of the spectrum and that their position and intensity is dependent upon the structure of the pigment. The spectrographic method thus allows the question of the inclusion of a pigment in the flavone series to be decided and may yield valuable results on further examination.

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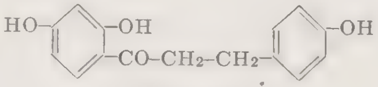
[40] Allan, Robinson: *J. Chem. Soc.*, **125**, 2192 (1924).

[41] Review and bibliography: Rupe, Schaerer in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 901; also Hayashi: *Acta phytochimica (Japan)*, **8**, 179 (1935); **9**, 1 (1936).

It has been suggested that there is a natural connection between sugars and flavones. Haworth[42] has shown that the cyclic structure of the normal carbohydrates corresponds to pyrane, and it may be imagined that the formation of these pigments[43] belonging to the C_{15} group takes place by the condensation of two molecules of hexose with one of triose. Phenols are certainly closely connected with the sugars, as is instanced by the production of pyrogallol and phloroglucinol by loss of water from inositol; and although compounds with 15 carbon atoms might also be constructed from isoprene units, it would seem that the hydroxyl groups in the pigments more probably originate as sugars. A further possibility would be the condensation of a phenolic aldehyde with acetaldehyde to yield a substituted cinnamic aldehyde, and the further condensation of this with a phenol. This combination would provide a compound with 15 carbon atoms, and the phenolic aldehyde might well arise from a hexose and a molecule of formaldehyde. Reduction of flavanones with zinc and acid gives flavopinacols. In particular the pinacol from 6:3′:4′-trihydroxyflavanone closely resembles natural phlobatannins.[41a]

Another suggestion connects the flavones with the chalkones as very closely related substances which are also known in nature, *e.g.*, phloretin:



Phloretin

occurs as a glycoside phloridzin in the root bark of fruit trees. Flavones would thus be regarded as derivatives of α-γ-diphenylpropane, and like the above they occur in nature almost invariably as glycosides. The glycosides are termed anthoxanthins, anthoxanthidins denoting the sugar-free pigments. The position of the sugar-residues can in many cases be determined by complete alkylation of the glycoside, followed by hydrolysis when the position in question appears as a free hydroxyl group; only the 3-, 5- and 7-hydroxyl groups seem to be concerned in glycoside formation. The anthoxanthins occur as solutions in the cell-sap of the parenchymatous tissues of cortex, leaves, flowers[44] and fruits as well as in wood.

Flavone pigments such as fisetin, luteolin, morin and quercetin were formerly extensively used as mordant dyes, particularly in the form of crude plant preparations (fustet, woad, fustic, Avignon berries, quercitron), although they now have been to a large extent displaced by synthetic dyestuffs.

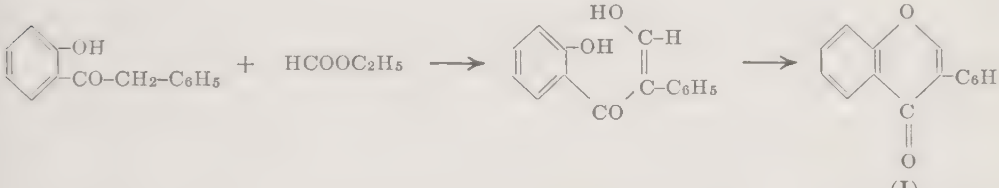
[41a] Russell, Clark: *J. Am. Chem. Soc.*, **61**, 2651 (1939).

[42] Haworth: *Ber.*, **65**, A, 44 (1932).

[43] Robinson: *Proc. Univ. Durham*, **8**, 14 (1927/32); Occurrence and distribution of the flavones: Hadders Wehmer in Klein, "Handbuch der Pflanzenanalyse," **III**, 2, p. 928.

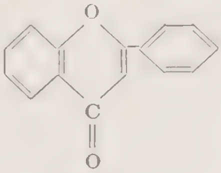
[44] Occurrence of flavones and flavonols in white flowers: Nakaoki: *J. pharm. Soc. (Japan)*, 52, 195 (1932); Distribution: Klein, Werner: *Z. physiol. Chem.*, **143**, 9 (1925).

The relation between anthoxanthidins, anthocyanidins (pigments of red and blue flowers) and catechin is considered later but it may be mentioned here that certain color reactions which serve for their recognition depend upon an interrelationship.[45] The isoflavone derivatives are of less importance and their chemistry has not been so thoroughly investigated. Their synthesis is effected by the interaction of formic ester with an appropriate phenyl benzyl ketone. Thus isoflavone[46] itself (I) is obtained:



(I)

Flavone,[47] $C_{15}H_{10}O_2$ (II) (colorless needles, m.p. 100°), was found by Müller as a flowery deposit on the leaves, flower-stems and seed-capsules of certain Primulae, which could be removed and crystallized. On fission with alkali, acetophenone, *o*-hydroxyacetophenone, salicylic and benzoic acids are obtained; and it may be synthesized among other ways from ω-benzal-*o*-hydroxyacetophenone.[48]



(II)

Dihydroxyflavones and -flavanones

Chrysin, (III), $C_{15}H_{10}O_4$ (5,7-dihydroxyflavone, yellow plates, m.p. 275°), is the yellow coloring matter of poplar buds and is also found as the glucoside toringin, $C_{21}H_{20}O_9$ (needles, m.p. 135-137°) in the bark of *Pirus Toringo.*[49] Acetophenone, phloroglucinol, acetic and benzoic acids

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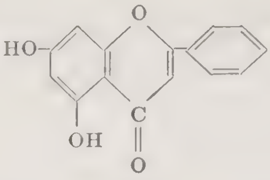
[45] Asahina. Inubuse: *Ber.*, 61, 1646 (1928); Shinoda: *J. Pharm. Soc. (Japan)*, 48, 35 (1928).

[46] Joshi, Venkataraman: *J. Chem. Soc.*, 1934, 513; the synthesis of the hydroxyderivatives, which is effected similarly, is mentioned under the individual natural compounds.

[47] Müller: *J. Chem. Soc.*, 107, 872 (1915). Older literature for all flavones: V. Mayer and P. Jacobson, "Lehrbuch der organischen Chemie," II, 3, p. 243.

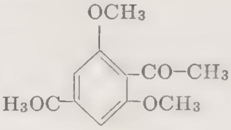
[48] Feuerstein, v. Kostanecki: *Ber.*, 31, 1757 (1898).

[49] Hirose: *J. Pharm. Soc. (Japan)*, 1909, 1.



(III)

are obtained on alkali treatment, and chrysin has been synthesized from phloracetophenone trimethylether



and ethyl benzoate[50] and also from phloroglucinol and the chloride of phenylpropiolic acid.[51]

Chrysin is accompanied in poplar buds by tectochrysin,[52] $C_{16}H_{12}O_4$, yellow needles (m.p. 165°). Tectochrysin is the 7-methyl ether of chrysin and was obtained from chrysin dimethyl ether and aluminum chloride, and also by the action of hydriodic acid and acetic anhydride on 2,4,6-trimethoxybenzoylacetophenone.* Chrysin is obtained from the fresh autumn or winter buds of the North American *Populus monilifera balsamifera* wherein it is particularly abundant (2-3 per cent by precipitating an alcoholic extract with lead acetate. Chrysin dyes wool mordanted with aluminum a pale yellow shade.

Primetin,[53] $C_{15}H_{10}O_4$ (yellow needles, m.p. 230-231°), occurs on the under side of the leaf of the Japanese *Primula modesta* (Bisset et Moore), accompanied by flavone, and is isolated by dissolving in alcohol. Alkaline hydrolysis yielded benzoic acid, no second fission product being obtained. It was at first thought to be 5,6-dihydroflavone, but the falsity of this assumption was proved by the synthesis of the 5,6-derivative. A synthetic 5-hydroxy-8-methoxyflavone has been identified with primetin monomethyl ether, so that primetin is 5:8-dihydroxyflavone.[53a]

Pratol,[54] (IV), $C_{16}H_{12}O_4$ (probably 7-hydroxy-4′-methoxyflavone, colorless needles, m.p. 261-262°), is found in the blossom of the red clover, *Trifolium pratense* or *Trifolium incarnatum*, from which it is obtained

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[50] Emilewicz, v. Kostanecki, Tambor: *Ber.*, **32**, 2448 (1899).

[51] Seka, Prosche: *Monatsh.*, **69**, 284 (1936); Hutchins, Wheeler: *J. Chem. Soc.*, **1939**, 91.

[52] Venkataraman, Bharadwaj: *Current. Sci.*, 2, 50 (1933); a pigment resembling tectochrysin in its color reactions is mentioned. Gulati, Venkataraman: *J. Chem. Soc.*, **1936**, 267.

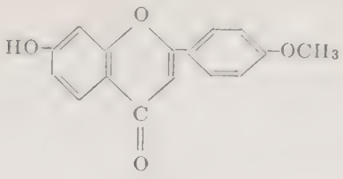
* The demethylation of methoxyflavones does not always proceed normally; some procedures lead to ring fission which is then followed by cyclization, so that flavones of misleading constitution are recovered.[52a]

[52a] Hattori: *Ber.*, **72**, 1914 (1939).

[53] Nagai, Hattori: *Acta phytochimica (Japan)*, **5**, 1 (1930); Sugasawa: *J. Chem. Soc.*, **1933**, 1621; Asahina: *Acta phytochimica (Japan)*, **7**, 187 (1933); Baker: *J. Chem. Soc.*, **1934**, 1953; **1939**, 956.

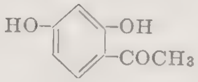
[53a] Baker, Brown, Scott: *J. Chem. Soc.*, **1939**, 1922.

[54] Power, Salway: *J. Chem. Soc.*, **97**, 231 (1910); Rogerson: *Ibid.*, **97**, 1006 (1910).



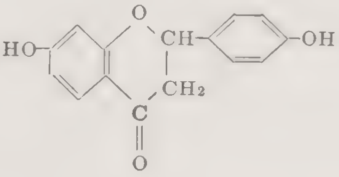
(IV)

by extraction with alcohol. A product closely resembling pratol has been obtained synthetically [55] from resacetophenone:



and benzoic anhydride.

Liquiritigenin,[56] (V), $C_{15}H_{12}O_4$ (7,4′-dihydroxyflavanone, colorless needles, m.p. 207°), occurs as the glucoside liquiritin, $C_{21}H_{22}O_9$ (colorless needles, m.p. 212°), in *Glycyrrhiza glabra* L. var. *gladifera* (Regel and Herder). The glucoside which is identical with the product of Fujita and Tsuda [57] isolated from the same species of *Glycyrrhiza,* is hydrolyzed into the above aglucone and glucose. The glucose residue is attached in position 4′, as after methylating the glucoside and opening the ring, paenol (1-acetyl-2-hydroxy-4-methoxybenzene) is obtained. Liquiritigenin itself, which forms a diacetate, is degraded by potassium hydroxide to resacetophenone and *p*-hydroxybenzoic acid. It has been synthesized from *p*-carbethoxycinnamic acid and resorcinol through 2,4,4′-trihydroxychalkone.



(V)

Trihydroxyflavones and -flavanones

Baicalein,[58] (I), $C_{15}H_{10}O_5$ (5,6,7-trihydroxyflavone, golden yellow needles, m.p. 264-265°), is contained in the root of *Scutellaria baicalensis* Georgi as baicalin, $C_{21}H_{18}O_{11}$ (yellow needles, m.p. 223°), which, according to its behavior toward cobaltic salts, is the 7-glycuronic acid derivative of the flavone. Fission of the trimethyl ether of baicalein gave

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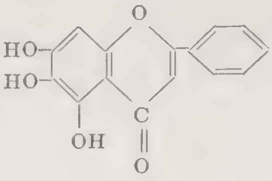
[55] Robinson, Venkataraman: *Ibid.*, 1926, 2344.

[56] Shinoda, Ueeda: *Ber.*, 67, 434 (1934).

[57] Lecture before the meeting of the pharmaceutical association, April, 1931.

[58] Shibata, Iwata, Nakamura: *Acta phytochimica (Japan)*, 1, 106 (1923); Shibata, Hattori: *Ibid.*, 5, 117 (1930); *J. Pharm. Soc. (Japan)*, 51, 15 (1931); Hattori: *Acta phytochimica (Japan)*, 5, 219 (1931); 5,6,7-trihydroxyflavone was synthesized prior to the discovery of baicalein: Bargellini: *Gazz. chim. ital.*, 49, *II*, 47 (1919); cf. Hattori: *Acta phytochimica (Japan)*, 5, 219 (1931).

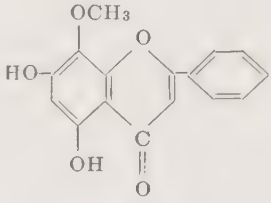


(I)

acetophenone, antiarol (3,4,5-trimethoxyphenol), and benzoic acid and synthesis[59] was effected from 2,4-dihydroxy-3,6-dimethoxyacetophenone and benzoic anhydride, followed by demethylation. Baicalein is obtained by hydrolyzing an alcoholic extract of the root, the yield being 4 per cent of the air-dried drug. Baicalein is used in China as a medicinal.

Oroxylin, isolated from the root-bark of *Oroxylium indicum* Vent.,[60] is a mixture of 5,7-dihydroxy-6-methoxyflavone and chrysin.

Wogonin,[61] (II), $C_{16}H_{12}O_5$ (5,7-dihydroxy-8-methoxyflavone, yellow needles, m.p. 203°), is present with baicalein in *Scutellaria baicalensis.*



(II)

It is also known as scutellarin[62] but must not be confused with the glycoside of scutellarein. Alkali fission[63] yields iretol (1,3,5-trihydroxy-2-methoxybenzene) and wogonin has been synthesized[64] in the usual way from benzoic anhydride and 2-hydroxy-3,4,6-trimethoxyacetophenone. It is prepared (yield 0.5 per cent of the air-dried material) by extracting the medicinal root "wogon" with benzene.

Apigenin, (III), $C_{15}H_{10}O_5$ (5,7,4′-trihydroxyflavone, pale yellow leaflets, m.p. 347-348°), is present as the glycoside apiin, $C_{26}H_{28}O_{14}$ (colorless needles, m.p. 228°), in parsley; apiin contains glucose and apiose, $C_5H_{10}O_5$, as carbohydrate constituents. Apigenin is hydrolyzed to phloroglucinol, *p*-hydroxyacetophenone and *p*-hydroxybenzoic acid, and has been synthesized from the trimethyl ether of phloracetophenone[65] and

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[59] Shah, Mehta, Wheeler: *Chem. Zentr.*, **1938,** II, 2114.

[60] Shah, Mehta, Wheeler: *Chem. Zentr.*, **1936,** I, 4576; **1936,** II, 2389; earlier literature; *J. Chem. Soc.*, **1936,** 591; **1938,** 1555; Bose, Bhattacharya: *Chem. Zentr.*, **1936,** I, 4576; **1939,** I, 136.

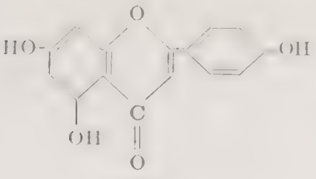
[61] Shibata, Iwata, Nakamura: *Acta phytochimica (Japan)*, **1,** 106 (1923); Shibata, Hattori: *J. Pharm. Soc. (Japan)*, **51,** 15 (1931); Hattori: *Acta phytochimica (Japan)*, **5,** 99 (1930); **5,** 219 (1931).

[62] Takahashi: *Mitteil. med. Fac. Tokyo*, **1,** 307 (1889).

[63] Hattori, Hayashi: *Ber.*, **66,** 1279 (1933).

[64] cf. Shah, Mehta, Wheeler: *Chem. Zentr.*, **1938,** II. 2114; *J. Chem. Soc.*, **1938,** 1555; Hutchins, Wheeler: *Chem. Zentr.*, **1939,** I, 664.

[65] Czajkowski, v. Kostanecki, Tambor: *Ber.*, **33,** 1988 (1900); Hutchins, Wheeler: *J. Chem. Soc.*, **1939,** 91.

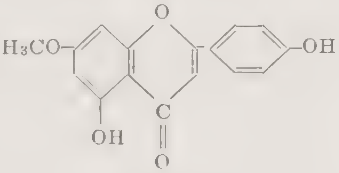


(III)

the methyl ester of anisic acid. Apigenin occurs also in the yellow dahlia [66] with small quantities of butin and has been isolated from Manchurian kaoliang, *Andropogon sorghum* Brot.[67]

Another glycoside of apigenin, cosmetin [68] is found in the flower-heads of *Cosmos bipinnatus* Cav. It has the formula $C_{21}H_{20}O_{16}$, forms yellow needles (m.p. 178°), and is apigenin-7-glucoside. One or more glycosides [69] of apigenin occur in the flowers of the field daisy *Chrysanthemum leucanthemum.* An apigenin glycoside occurs in *Zinnia elegans* Jacq.[69a] Apigenin, which possesses somewhat greater tinctorial power than chrysin, is obtained by purifying an alcoholic extract of parsley and hydrolyzing the apiin (0, 1-0, 2 per cent) so obtained.

Genkwanin,[70] (IV), $C_{16}H_{12}O_5$ (5,4′-dihydroxy-7-methoxyflavone, pale-yellow needles, m.p. 286°), occurs with apigenin in "genkwa," a drug which was being used medicinally in China in 160 B.C. It contains one methoxy and two hydroxyl groups, yields *p*-hydroxybenzoic acid and phloroglucinol with fused potash, and *p*-hydroxyacetophenone and phloroglucinol monomethyl ether with 50 per cent potassium hydroxide. Genkwanin was synthesized [71] from phloracetophenone and ethyl-*p*-benzoyloxybenzoate. An alcoholic extract of the blooms is a convenient source of genkwanin.



(IV)

Acacetin,[72] (V), $C_{16}H_{12}O_5$ (5,7-dihydroxy-4′-methoxyflavone, colorless needles, m.p. 261°), is present as the rhamnoside acaciin, $C_{28}H_{32}O_{13}$

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[66] Schmid, Waschkau: *Monatsh.*, 49, 83 (1928); Schmid, Seebald: *Monatsh.*, 60, 32 (1932); Schmid, Haschek: *Monatsh.*, 62, 317 (1933); Price: *J. Chem. Soc.*, 1939, 1017.

[67] Okano, Abe, Ohara: *Chem. Zentr.*, 1935, II, 1046.

[68] Nakaoki: *J. Pharm. Soc. (Japan)*, 55, 173 (1935).

[69] Jacini: *Helv. Chim. Acta*, 18, 276 (1935).

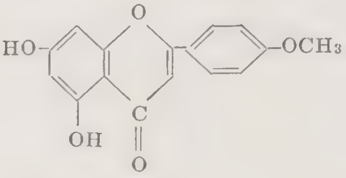
[69a] Nakaoki: *J. Pharm. Soc. Japan*, 60, 502 (1940).

[70] Nakao, Tseng: *J. Pharm. Soc. (Japan)*, 52, 83, 148 (1932).

[71] Tseng: *Ibid.*, 55, 30 (1935); Mahal, Venkataraman: *J. Chem. Soc.*, 1936, 569.

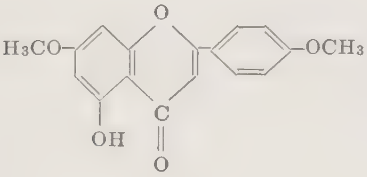
[72] A. G. Perkin: *J. Chem. Soc.*, 77, 423 (1900); Hattori: *Acta phytochimica (Japan)*, 2, 99 (1925); Robinson, Venkataraman: *J. Chem. Soc.*, 1926, 2344.

(colorless needles, m.p. 260°), in false acacia, *Robinia pseudacacia.* Hydrolysis products are phloroglucinol and *p*-hydroxybenzoic acid, and a synthesis from phloracetophenone and anisic anhydride has been carried out. Acacetin is precipitated from an aqueous extract of the leaves with basic lead acetate.

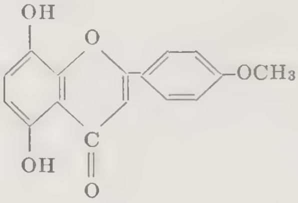


(V)

5-Hydroxy-7,4′-dimethoxyflavone,[73] (VI), $C_{17}H_{14}O_5$, is a pigment obtained in a yield of about 3 g/kg by extracting the buds of the birch with alcohol after a preliminary extraction with petroleum ether. Fission gave anisic acid, and demethylation afforded apigenin:



(VI)



(VII)

5,8-Dihydroxy-4′-methoxyflavone,[74] (VII), $C_{16}H_{12}O_5$ [yellow needles, m.p. 240° (still slightly impure)], occurs in the leaves of *Gingko bilota* (maidenhair tree). The compound gives flavone color reactions, *e.g.,* a red coloration with magnesium and hydrochloric acid in the presence of mercury, but is not identical with acacetin. Fusion with potash yields *p*-hydroxybenzoic acid, and 40-per cent potassium hydroxide gives *p*-hydroxyacetophenone, anisic acid and phloroglucinol. With diazomethane only a monomethyl ether is formed, but this gives a monoacetyl compound. Spectrographic examination excludes position 6 for the methoxy group and suggests that this is on carbon atom 4′. The pigment is obtained by a somewhat involved method from an alcoholic extract of the leaves.

Galangin, (VIII), $C_{15}H_{10}O_5$ (3,5,7-trihydroxyflavone, yellow tablets, m.p. 219-221°), occurs in galanga root (rhizome of *Alpinia officinarum*) together with kaempheride. Galangin is degraded to phloroglucinol and benzoic acid and has been synthesized[75] from benzaldehyde and the trimethyl ether of phloracetophenone and from phloroglucinol and benzoyloxyacetonitrile. It dyes a yellow shade. Galangin is obtained by working up the mother-liquors from the separation of kaempferide. The monomethyl ether,[76] synthesized from benzoic anhydride and ω-methoxyphloracetophenone occurs with galangin in kaempheride.

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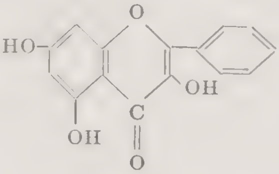
[73] Bauer, Dietrich: *Ber.,* 66, 1053 (1933).

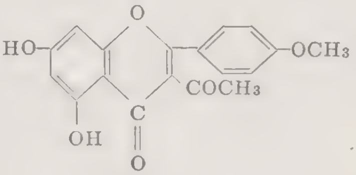
[74] Furukawa: *Bull. Inst. Phys. Chem. Research (Tokyo),* 2, 5 (1929); *Sci. Papers Inst. Phys. Chem. Research (Tokyo),* 19, 27 (1932); 21, 278 (1933).



(VIII)

Izalpinin[77] is obtained from the Japanese drug Izu-syuku-sya in addition to alpinone[78]; with the formula $C_{16}H_{12}O_5$ (yellow needles, m.p. 195°), it is the 7-methyl ether of galangin, a view which has been confirmed by synthesis.[79]

Buddleoflavonol,[80] (IX), $C_{18}H_{14}O_6$ (3-acetyl-4′-methoxy-5,7-dihydroxyflavone, yellow needles, m.p. 265°) is contained in the flowers and foliage of *Buddleia variabilis* as the glycoside buddleoflavonoloside, $C_{30}H_{34}O_{15}$ (light-yellow needles, m.p. **274-276°**). The glycoside contains, in addition to the above aglucone, the units of rhamnose and glucose although their points of attachment are not known. Fission of buddleoflavonol gives phloroglucinol, anisic acid and acetone but the structural formula has not yet been established beyond doubt.



(IX)

Citronetin,[81] (X), $C_{16}H_{14}O_5$ (5,7-dihydroxy-2-methoxy-flavanone, colorless plates, m.p. 224-225°) is found in the peel of *Citrus limon* Burm. f. *Ponderosa* Hort., as the glycoside citronin. Citronetin has been synthesized from phloroglucinol and *o*-methoxycinnamic acid.

[75] Chavan, Robinson: *J. Chem. Soc.*, **1933**, 368, a synthesis which served as a preliminary to the synthesis of flavonol-glycosides; synthesis of a glycoside, Brit. P. 490,360 (I. G.), *Chem. Zentr.*, **1939**, I, 2085.

[76] Testoni: *Gazz. chim. ital.*, **30**, II, 327 (1900); Kalff, Robinson: *J. Chem. Soc.*, **127**, 181 (1925).

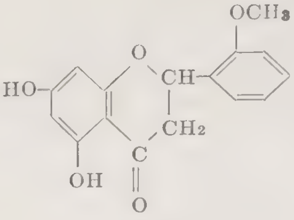
[77] Kimura, Hoshi: *J. Pharm. Soc. (Japan)*, **54**, 135 (1934).

[78] Kimura, Hoshi: *Chem. Zentr.*, **1937**, I, 3809.

[79] Kimura Hoshi: *J. Pharm. Soc. (Japan)*, **55**, 229 (1935).

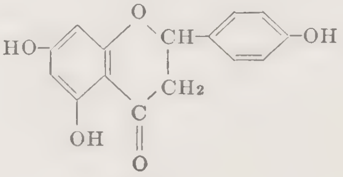
[80] Yü: *Bull. soc. chim. biol.*, **15**, 482 (1933).

[81] Yamamoto, Oshima: *J. agri. chem. soc. Japan*, **1931**, No. 79, 312; Shinoda, Sato: *J. Pharm. Soc. Japan*, **51**, 78 (1931).



(X)

Naringenin,[82] (XI), $C_{15}H_{12}O_5$ (5,7,4′-trihydroxyflavanone, colorless needles, m.p. 251°) occurs in the flowers and fruits of *Citrus decumana* (shaddock) in the form of the rhamno-glycoside, naringin, $C_{27}H_{32}O_{14}$ (needles, m.p. 82°), in which the sugar residue is located in position 7. Naringenin decomposes by alkaline fission into phloroglucinol and *p*-cumaric acid and has been synthesized [83] from these compounds. It is obtained [84] by extracting the skins of the fruits with alcohol and purifying and hydrolyzing the naringin.



(XI)

Sakuranetin,[85] (XII), $C_{16}H_{14}O_5$ (5,4′-dihydroxy-7-methoxyflavanone, colorless needles, m.p. 150°), is present in *Prunus yedoensis* and in the variety *Prunus serrulata* as the glycoside sakuranin, $C_{22}H_{24}O_{10}$ (colorless needles, m.p. 212°); the latter is converted by barium hydroxide into hydroxybenzaldehyde and a second glycoside, $C_{15}H_{20}O_9$, which on further hydrolysis yields glucose and phloracetophenone-4-monomethyl ether. Sakuranin may be hydrolyzed into sakuranetin and glucose, however, by dilute sulfuric acid. The synthesis of sakuranetin [83] follows that of naringenin. It would appear to be most conveniently isolated by extracting the root-bark of *Prunus serrulata* Lindl. var. *albida Makino subv. speciosa Makino* with methyl alcohol. Yield 1.8 per cent.

Isosakuranetin,[86] (XIII), formerly termed kikokunetin, $C_{16}H_{14}O_5$ (5,7-dihydroxy-4′-methoxyflavanone, colorless needles, m.p. 194-195°), occurs

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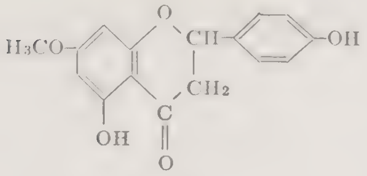
[82] Asahina, Inubuse: *Ber.*, **61**, 1514 (1928); Asahina, Shinoda, Inubuse: *J. Pharm. Soc. (Japan)*, **48**, 29 (1928); Asahina, Asano, Inubuse: *Ibid.*, **49**, 11 (1929); the conversion of naringin into isosakuranetin is described; Shinoda, Sato: *J. Pharm. Soc. (Japan)*, **48**, 117 (1928); Pulley: *Chem. Zentr.*, **1938**, I, 4665.

[83] K. Rosenmund, M. Rosenmund: *Ber.*, **61**, 2608 (1928).

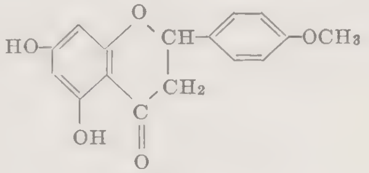
[84] Detection in the bark of the peach tree: Shinoda, Uyeda: *J. Pharm. Soc. (Japan)*, **49**, 97 (1929); Isolation: Poore: *Ind. Eng. Chem.*, **26**, 637 (1934).

[85] Asahina, Shinoda, Inubuse: *J. Pharm. Soc. (Japan)*, **1927**, 133; Shinoda, Sato: *Ibid.*, **48**, 33 (1928); Asahina, Inubuse, Asano: *Ibid.*, **49**, 11 (1929); *cf.* Asahina, Shinoda, Inubuse: *Ibid.*, **48**, 29 (1928).

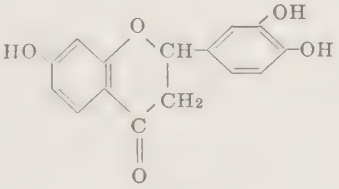
[86] Hattori: *Ibid.*, **48**, 144 (1928); *Acta phytochimica (Japan)*, **4**, 219 (1929); Shinoda: *J. Pharm. Soc. (Japan)*, **48**, 173 (1928); Shinoda, Sato: *Ibid.*, **48**, 109 (1928); Asahina, Inubuse, Asano: *Ibid.*, **49**, 11 (1929).


(XII)

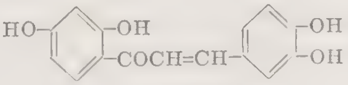
in *Pseudaegle trifoliata Makino,* one of the *Rutiaceae.* Fusion with potash yields phloroglucinol and anisic acid, and it has also been synthesized from phloroglucinol and *p*-methoxycinnamic acid. It is isolated by extracting the blossom with alcohol, 600 of the air-dried flowers yielding 2 grams of pure material.


(XIII)

Butin,[87] $C_{15}H_{12}O_5$ (7,3′,4′-trihydroxyflavanone, colorless needles, m.p. 224-226°), is found as the glycoside butrin, $C_{27}H_{32}O_{15}$ (silky needles) in


Butin

the flowers of *Butea frondosa,* and is identical with the Indian material known as tesu. Butin is converted by ring-fission on boiling with potassium hydroxide into the chalkone butein:


Butein

The reverse change takes place in alcoholic sulfuric acid. On boiling with 50-per cent potassium hydroxide butein breaks down into resacetophenone and protecatechuic acid. It has been synthesized[88] from 2,3-dicarboethoxycinnamic acid chloride and resacetophenone:

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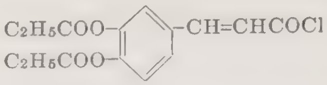
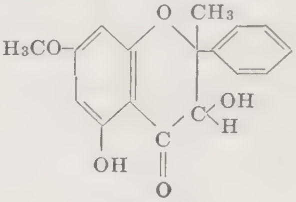
[87] Lal: *Chem. Zentr.,* 1937, II, 77.

[88] Shinoda, Sato, Kawagoye: *J. Pharm. Soc. (Japan),* 49, 123 (1929); cf. the comments of Göschke, Tambor: *Ber.,* 44, 3502 (1911), on the synthesis from protocatchuic aldehyde.



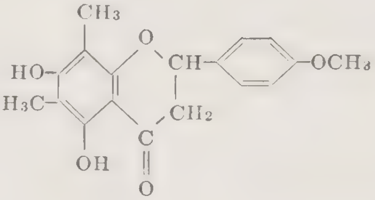
A yield of 2 per cent of butin is obtained by extracting the flowers with boiling water and hydrolyzing the glycoside.

Alpinone,[89] (XIV), $C_{17}H_{16}O_5$ (colorless needles, m.p. 178°), found in the Japanese drug Izu-Syuku-sya, is constituted as 3,5-dihydroxy-7-methoxy-2-methylflavanone, a formulation confirmed by the formation of 2-hydroxy-4,6-α-trimethoxy-β-methylchalkone on submitting the dimethyl ether to ring fission, and by synthesis.

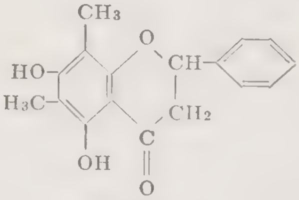


(XIV)

Mattecinol,[90] (XV), $C_{18}H_{18}O_5$ (5,7-dihydroxy-6,8-dimethyl-4′-methoxyflavanone, colorless needles, m.p. 174°), occurs in the leaves of *Matteucia orientalis.* Fusion with potash yields 2,4-dimethylphloroglucinol and p-methoxycinnamic acid, from which two compounds it may be resynthesized. It is accompanied by desmethoxymatteucinol, $C_{17}H_{16}O_4$ (pale yellow plates, m.p. 200°); this compound, 5,7-dihydroxy-6,8-dimethylflavanone (I), has been synthesized from 2,4-dimethylphloroglucinol and cinnamic acid. Matteucinol and desmethoxymatteucinol (XVI) are the



(XV)



(XVI)

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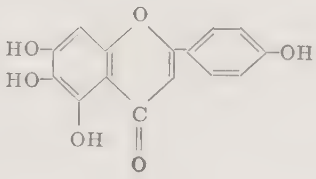
[89] Kimura, Hoshi: *Chem. Zentr.*, **1937**, I, 3809.

[90] Munesada: *J. Pharm. Soc. (Japan)*, **1924**, 12; Fujise: *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **11**, 111 (1929); Fujise, Nishi: *Ber.*, **66**, 929 (1933); Fujise, Kubota: *Ber.*, **67**, 1905 (1934).

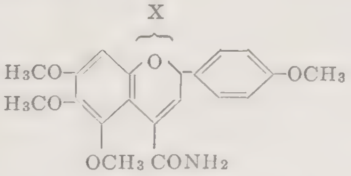
only optically active flavone derivatives known to occur in nature,[91] $[\alpha]^{28}_{D} = -39.47°$ and $-50°$ (acetone) respectively. Differences in melting point between the synthetic and natural products find an explanation in this circumstance and disappear on racemizing the natural compounds. The compounds are obtained by extracting the leaves with acetone, but their separation and purification is hindered by the formation of mixed crystals.

Tetrahydroxyflavones and -flavanones

Scutellarein,[92] (I), $C_{15}H_{10}O_6$ (5,6,7,4′-tetrahydroxyflavone, yellow needles, m.p. 330-350°), occurs in the glycoside scutellarin, $C_{21}H_{18}O_{12}$ (yellow needles, m.p. $> 312°$), in *Scutellariae* varieties in combination with glycuronic acid; this scutellarin is not to be confused with the compound scutellarin which is identical with wogonin. Scutellarein is also found in *Galeopsis Tetrahit* and *Teucrium Chamaedrys.* Fission yields phloroglucinol, *p*-hydroxyacetophenone and *p*-hydroxybenzoic acid, and synthesis[93] may be effected from the methyl ester of anisic acid and 2,4-dihydroxy-3,6-dimethoxyacetophenone. The latter synthesis does not completely establish the structure but the condensation of the amide of anisoyl pyruvic acid,[94] $H_3CO—C_6H_4—CO—CH_2—CO—COOH$, with antiarol to the flavylium salt:



(I)



removed the ambiguity, as this was converted into the amine and thence into the tetramethyl ether of the flavone which was identical with scutellarein tetramethyl ether. Scutellarein is conveniently obtained by extracting *Scutellaria altissima* or *indica* with water.

Luteolin,[95] (II), (digitoflavone), $C_{15}H_{10}O_6$ (5,7,3′,4′-tetrahydroxyflavone, yellow needles, m.p. 329-330°), is present in the formerly widely used wild woad (*Reseda luteola*). A glycoside galuteolin, $C_{21}H_{20}O_{11}$ (yellow needles, decomposing at 280°) occurs in the seeds of *Galega officinalis,* and a 7-glucoside, $C_{21}H_{20}O_{11}$ (m.p. 258°) occurs in digitalis[96] leaves. Luteolin is found also in dyer's broom (*Genista tinctoria* L.). Significant fragments obtained on fission are phloroglucinol, 3,4-dihydroxyacetophenone and protocatechuic acid, and synthesis[97] has been effected from the ethyl ester of veratric acid (ethyl 3,4-dimethoxybenzene-1-carboxylate) and phloracetophenone trimethyl ether.

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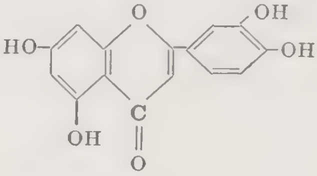
[91] Resolution: Fujise, Nagasaki: *Ber.*, 69, 1893 (1936).
[92] Wessely, Moser: *Monatsh.*, 56, 97 (1930).
[93] Bargellini: *Gazz. chim. ital.*, 45, I, 69 (1915): 49, II, 47 (1919).
[94] Robinson, Schwarzenbach: *J. Chem. Soc.*, 1930, 822.
[95] Literature: Schultz: "Farbstofftabellen," 7 ed., I, p. 631, No. 1371.



(II)

The *Reseda* plant was formerly cultivated and plucked and dried after one year. The pigment was an important orange-yellow dye and was used as long ago as the time of Julius Caesar, but today is used only for dyeing silk with aluminum or tin mordants. It is obtained from commercial woad extract. A flavonol glycoside, $C_{27}H_{30}O_{15} \cdot 2H_2O$, m.p. 223-224°, occurs in the leaves and stems of *Calystegia japonica* Chois. Hydrolysis gave glucose, rhamnose, and an aglucone $C_{15}H_{10}O_6$, m.p. 272-274°. As methylation of the glycoside followed by hydrolysis afforded 3-hydroxy-4′:5:7-trimethoxyflavone, m.p. 151°, it would seem probable that the natural compound is a glycoside of 3:4′5:7-tetrahydroxyflavone with the sugar residues attached to the 3-position.[97a]

Diosmetin,[98] (III), (formerly termed hyssopin), $C_{16}H_{12}O_6$ (5,7,3′-trihydroxy-4′-methoxyflavone, yellow needles, m.p. 253-255°), occurs as the glycoside diosmin, which is the 7-rhamnoglycoside, $C_{28}H_{32}O_{15}$ (yellow crystals, m.p. 278°) in hyssop plants (for individual varieties see Oesterle and Wander) and in *Dahlia variabilis.* Diosmetin is broken down by caustic potash into acetovanillone (1-aceto-3-hydroxy-4-methoxybenzene), isovanillic acid (3-hydroxy-4-methoxybenzene-1-carboxylic acid) and phloroglucinol; and it may be demethylated to luteolin. It has been synthesized[99] from phloracetophenone and *o*-benzoylisovanillic acid. Hydroxyapiin methyl ether, the 7-apiose glycoside[100] of diosmetin, has been recognized from its fission fragments but has not so far been isolated.

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[96] Fleischer, Fromm: *Ber.*, 32, 1184 (1899); Diller, v. Kostanecki: *Ber.*, 34, 1453 (1901); Kiliani, Mayer: *Ber.*, 34, 3577 (1901); Nakamura, Ohta, Hukuti: *J. Pharm. Soc. (Japan)*, 56, 107 (1936).

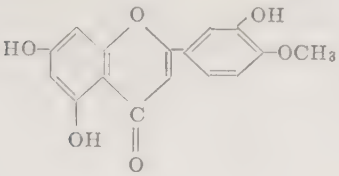
[97] v. Kostanecki, Różycki, Tambor: *Ber.*, 33, 3410 (1900).

[97a] Hukuti: *J. Pharm. Soc. Japan*, 59, 258 (1939).

[98] Oesterle, Wander: *Helv. Chim. Acta*, 8, 519 (1925); Shriner, Kleiderer: *J. Am. Chem. Soc.*, 51, 1267 (1929); Shinoda, Ueyeda, Sato: *J. Pharm. Soc. (Japan)*, 50, 65 (1930); Nakaoki: *Ibid.*, 58, 197 (1938).

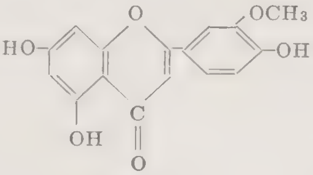
[99] Lovecy, Robinson, Sugasawa: *J. Chem. Soc.*, 1930, 817.

[100] Rupe, Schaerer in Klein, "Handbuch der Pflanzenanalyse," III, 2, p. 863.



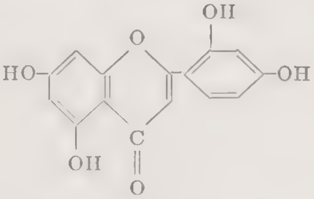
(III)

Chrysoeriol,[101] (IV), $C_{16}H_{12}O_6$ (5,7,4′-trihydroxy-3′-methoxyflavone, yellow needles, m.p. 324-325°), occurs in the leaves (Yerba Santa) of the Californian *Hydrocephyllacea Erydictyon glutinosum* Benth. It has been synthesized from phloracetophenone and *o*-benzylvanillic acid (m.p. 328-330°). Chrysoeriol is obtained by a somewhat troublesome procedure from an alcoholic extract of the leaves.



(IV)

Lotoflavin,[102] (V), $C_{15}H_{10}O_6$ (5,7,2′,4′-tetrahydroxyflavone, yellow plates which blacken at 300°), occurs as its glycoside lotusin, $C_{28}H_{31}O_{16}N$ (pale-yellow needles) in the sap of *Lotus arabicus*. The sugar component is the cyanhydrin of maltose and the glycoside itself may be hydrolyzed to maltose, hydrocyanic acid and lotoflavin; the latter on more drastic fission yields phloroglucinol and β-resorcyclic acid (2,4-dihydroxybenzene-1-carboxylic acid) and may be obtained synthetically from phloracetophenone-4,6-dimethyl ether and the methyl ester of 2,4-dimethoxybenzoic acid via 2-hydroxy-4,6,2′,4′-tetramethoxybenzoylacetophenone. On extracting with methyl alcohol 1 kg of dried blooms yields 25 grams of lotusin, which is then hydrolyzed.



(V)

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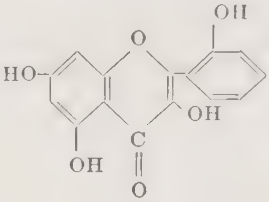
[101] Tutin, Clewer: *J. Chem. Soc.*, 95, 81 (1909); Oesterle: *Arch. Pharm.*, 256, 119 (1918); Lovecy, Robinson, Sugasawa: *J. Chem. Soc.*, 1930, 817.

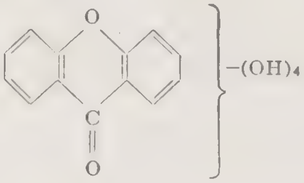
[102] Dunstan, Henry: *Proc. Roy. Soc. London*, 68, 374 (1901); *Chem. News*, 84, 26 (1901); Cullinane, Algar, Ryan: *Sci. Proc. Roy. Dublin Soc.*, 19, 77 (1928); cf. also Henry: *J. Soc. Chem. Ind.*, 57, 248 (1938).

Datiscetin,[103] (VI), $C_{15}H_{10}O_6$, (3,5,7,2′-tetrahydroxyflavone, yellow needles, m.p. 276°), is found in the roots, leaves and branches of *Datisca cannabina* as the glycoside datiscin, $C_{27}H_{30}O_{15}$ (needles or leaflets, m.p. 192-193°). The glycoside may be hydrolyzed into datiscetin and rutinose, a disaccharide containing the units of rhamnose and glucose. Alkaline fission of datiscetin yields salicyclic acid, and by the action of bromine in acetic acid tribromphloroglucinol is obtained. A synthesis [104] of datiscetin has been effected from ω-methoxyacetophenone and *o*-methoxybenzoic acid.



(VI)

The many discrepancies in the literature relating to datiscetin may be traced to the occurrence of two colored compounds. The second to be isolated has the composition corresponding to $C_{15}H_{12}O_6$ (yellow needles, m.p. 237°); this yields salicylic acid on fusing with potash, and 5-nitro-2-hydroxybenzoic and picric acids on treating with dilute and concentrated nitric acids, respectively. It contains two hydroxyl groups but passes on demethylation into a yellow compound, $C_{13}H_8O_6$ (m.p. 260°), which is formulated as a tetrahydroxyxanthone:



so that the original coloring matter is a dimethyl derivative of this compound. On the other hand, examination of older preparations of Schunck (m.p. 237°) would suggest the occurrence of galangin. Datiscetin is used as a yellow dye for silk. It is prepared from an alcoholic extract of the root and a yield of about 4 per cent is obtained.

Kaempferol, (VII), $C_{15}H_{10}O_6$ (3,5,7,4′-tetrahydroxyflavone, yellow needles, m.p. 276-278°), occurs as glycosides, as in robinin, $C_{33}H_{40}O_{15}$ (yellow needles, m.p. 196-197°), from *Robinia pseudacacia.* Robinin[105] is the 3-robinoside, the robinose being further hydrolyzable to 1 mol. of galactose and 2 mols. of rhamnose. Kaempferol occurs also in *Rosa multiflora,* as multiflorin, $C_{27}H_{30}O_{15}$ (yellow needles with an indistinct m.p. 147-170°), a rhamnoside used in Japan as a laxative under the name Eijitsu.[106] Other glycosides known are a rhamnoside in the flowers of *Acacia* varieties,[107] kaempferin, $C_{27}H_{30}O_{16}$ (yellow needles, m.p. 185-186°), from *Cassia augustifolia*[108] and containing two glucose units; a glucose derivative from *Hortensia* blooms; kaempferitrin (indigo yellow), a 3-rhamnoside, $C_{27}H_{30}O_{14}$ (colorless needles, m.p. 201-203°), found in dyer's knot-grass, and *Indigofera arrecta.* A glycoside,[109] $C_{27}H_{30}O_{15}$ (yellow needles, m.p. 223-224°), found in leaves and stalks of *Calystegia japonica* Chais, is broken down into kaempferol, rhamnose and glucose.

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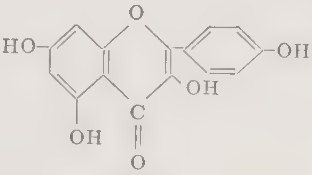
[103] Braconnot: *Ann. chim. (2),* 3, 277 (1816); Stenhouse: *Ann.*, **98**, 167 (1856); Schunck, Marchlewski: *Ann.*, **277**, 261 (1893); **278**, 351 (1894); Charaux: *Compt. rend.*, **180**, 1419 (1925); Korczyński, Marchlewski: *Chem. Zentr.*, **1906**, II, 1265; **1907**, II, 700; *Biochem. Z.*, **3**, 295 (1907); Leskiewicz, Marchlewski: *Ber.*, **47**, 1599 (1914).

[104] Bargellini, Peratoner: *Gazz. chim. ital.*, **49**, II, 64 (1919); Kalff, Robinson: *J. Chem. Soc.*, **127**, 1968 (1925).



(VII)

Again, the fruits of *Sophora japonica* L. contain sophoricoside, rutoside and sophoroflavonoloside,[110] the last of which yields kaempferol on fission. Kaempferol is also found in *Delphinium consolida, Prunus spinosa, Crocus asturicus, Crocus speciosus,*[111] in senna leaves, in the berries of *Rhamnus catharticus* and accompanying indigo. Kaempferol is broken down on fission into phloroglucinol and *p*-hydrobenzoic acid and has been synthesized[112] from anisaldehyde and phloracetophenone dimethyl ether. It is conveniently prepared from the commercial extract of the bloom of *Delphinium consolida.*

Lespedin, $C_{27}H_{30}O_{13}$, yellow needles (hydrate, m.p. 234° from aqueous alcohol, or m.p. 193° from water) is obtained from *Lespedeza cyrtobotrya.* It forms both mono- and dimethyl ethers. Lespedin is a 3:7-birhamnoside of kaempferol and is thus probably identical with kaempferitrin.[113]

Kaempferide, (VIII), $C_{16}H_{12}O_6$ (3,5,7-trihydroxy-4′-methoxyflavone, yellow needles, m.p. 227-229°), is found in Galanga root, the rhizome of

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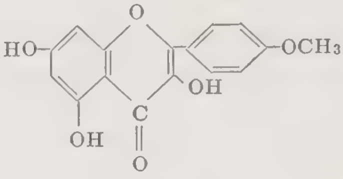
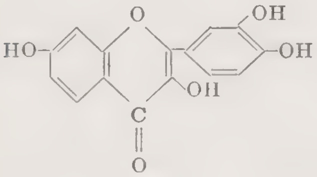
[105] Charaux: *Bull. soc. chim. biol.,* **8,** 915 (1926); Sando: *J. biol. chem.,* **94,** 675 (1931/32).
[106] Kondo, Iwamoto, Kuchiha: *J. Pharm. Soc. (Japan),* **49,** 35 (1929); Kondo, Endo: *Ibid.,* **49,** 182 (1929).
[107] Petrie: *Biochem. J.,* **18,** 957 (1924).
[108] Tutin: *J. Chem. Soc.,* **103,** 2006 (1913).
[109] Hukuti: *J. Pharm. Soc. (Japan),* **59,** 85 (1939).
[110] Rabaté, Dussy: *Bull. soc. chim. biol.,* **20,** 459, 467 (1938).
[111] Price, G. M. Robinson, R. Robinson: *J. Chem. Soc.,* **1938,** 281.
[112] v. Kostanecki, Lampe, Tambor: *Ber.,* **37,** 2096 (1904).
[113] Hattori, Hasegawa: *Proc. Imp. Acad. Tokyo,* **116,** 9 (1940).

Alpinia officinarum, from the commercial alcoholic extract of which it may be prepared.

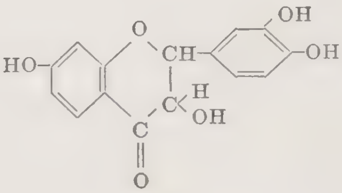


(VIII)

Fisetin,[113a] (IX), $C_{15}H_{10}O_6$ (3,7,3′,4′-tetrahydroxyflavone, yellow prisms, m.p. 330°), occurs as the glycoside tannic acid fustin tannide (yellowish needles, m.p. 200°) in the heart-wood of the sumach tree (*Rhus cotinus*) and in the wood of *Quebracho colorado.* After removing the tannic acid a product, fustin (X),[114] $C_{15}H_{12}O_6$ (yellowish needles, m.p. 218-219°), is obtained which is a dihydrofisetin:



(IX)



(X)

A second glycoside forming colorless needles (m.p. 215-217°) is found in the wood of the yellow cedar (*Rhus rhodanthema*). Fisetin is decomposed into resorcinol and protocatechuic acid and has been synthesized [115] from veratric aldehyde and resacetophenone ethyl ether.

The woods containing fisetin come into commerce under the names of fiset-wood, fustic, young fustic, fustel and Hungarian yellow-wood, and the dried extract obtained with soda solution is known as cotinin. The pigment finds limited application in the dyeing of wool and as an orange or scarlet dye for leather. The dyeings possess considerable stability to fulling and alkalies (soaps), but are not fast to light.

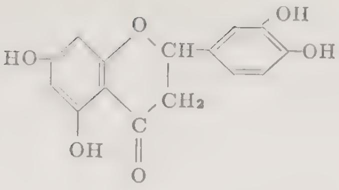
Eriodictyol,[116] (XI), $C_{15}H_{12}O_6$ (5,7,3′,4′-tetrahydroxyflavanone, colorless needles, m.p. 267°), occurs in one of the *Hydrocephyllaceae Eriodictyon glutinosum* Benth., together with its 3′-methyl ether homoeriodictyol (XII), $C_{16}H_{14}O_6$ (yellowish tablets, m.p. 224-225°).

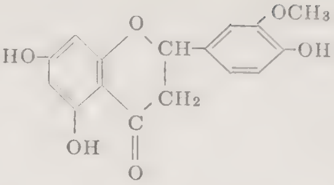
[113a] Literature: Schultze, "Farbstofftabellen," 7 Ed., I, p. 626, No. 1365.

[114] Oyamada: *Ann.*, **538**, 44 (1939).

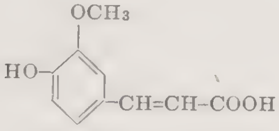
[115] v. Kostanecki, Lampe, Tambor: *Ber.*, **37**, 784 (1904).

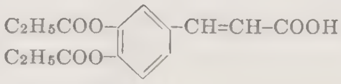
[116] Shinoda, Sato: *J. Pharm. Soc. (Japan)*, **49**, 5, 7 (1929); Russell, Todd: *J. Chem. Soc.*, **1937**, 421; Seka, Prosche: *Monatsh.*, **69**, 284 (1936); Synthesis of a glycoside: Brit. P. 490.360 (I. G.). *Chem. Zentr.*, **1939**, I, 2085. Improved extraction of eriodictyol and homoeriodictyol: Geissmann: *J. Am. Chem. Soc.*, **62**, 3258 (1940).


(XI)


(XII)

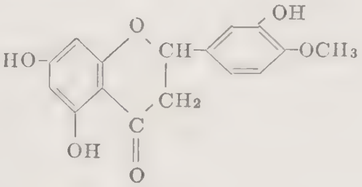
Homoeriodictyol undergoes fission into phloroglucinol and ferulic acid (XIII). Eriodictyol has been synthesized from phloroglucinol and 3,4-dicarbethoxycinnamic acid (XIV) and homoeriodictyol from phloroglucinol and carbethoxyferulic acid.

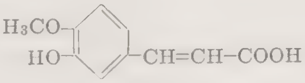

(XIII)


(XIV)

Both compounds are obtained from an alcoholic extract of the leaves.

Hesperitin,[117] (XV), $C_{16}H_{14}O_6$ (5,7,3′-trihydroxy-4′-methoxyflavanone, yellow plates, m.p. 226°), occurs as the 7-rhamnoside hesperidin, $C_{28}H_{34}O_{15}$ (colorless needles, m.p. 252°), in *Citrus* varieties, *e.g.*, in the lemon and orange. Neohesperidin[118] (m.p. 244°), occurring in the unripe orange, is hydrolyzed to rhamnose and glucohesperidin, $C_{22}H_{24}O_{11}$, which may be further broken down into glucose and hesperidin. Neohesperidin is thus a diglycoside. The bark of the peach tree contains persicoide,[119] $C_{22}H_{24}O_{11}$ (needles, m.p. 250°), which contains the units of glucose and "hesperetol" = hesperitin. Alkaline fission of hesperitin yields phloroglucinol and isoferulic acid:


(XV)



but a synthesis has not yet been accomplished. Hesperidin is obtained from unripe bitter oranges which are extracted with cold water.

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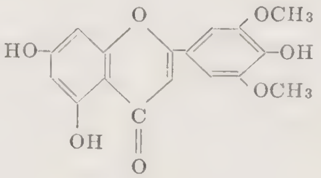
[117] Asahina, Shinoda, Inubuse: *J. Pharm. Soc. (Japan)*, **48**, 29 (1928); Asano, Asahina, Inubuse: *Ibid.*, **49**, 11 (1929); King, Robertson: *J. Chem. Soc.*, **1931**, 1704; Tseng, Yu: *Chem. Zentr.*, 1937, I. 2186; Seka, Prosche: *Monatsh.*, **69**, 284 (1936) (m.p. 233°).

[118] Kolle, Gloppe: *Pharm. Zentralhalle*, **77**, 421 (1936).

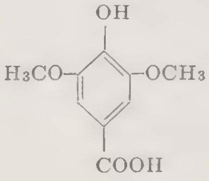
[119] Charaux, Rabaté: *J. Pharm. Chim. (8)*, **21**, 495 (1935); *Compt. rend.*, **200**, 1689 (1935).

Pentahydroxyflavones

Tricin,[120] (I), $C_{17}H_{14}O_7$ (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, pale yellow needles, m.p. 288°), is found in *Triticum dicoccum.* Fission yields phloroglucinol and syringic acid (II):



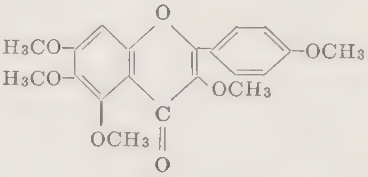
(I)



(II)

The parent 5,7,3′,4′,5′-pentahydroxyflavone, which bears the name tricetin, has been synthesized from 3,4,5-trimethoxybenzoic anhydride and phloracetophenone with subsequent demethylation; and another synthesis [121] from phloracetophenone and *o*-benzylsyringic acid led finally to the preparation of tricin. Tricin dyes wool a pale yellow shade on an aluminum mordant. It is prepared from an alcoholic extract of the leaves.

Tangeretin,[122] (III), $C_{20}H_{20}O_7$ (3,5,6,7,4′-pentamethoxyflavone, colorless needles, m.p. 154°), is found in the expressed sap of the rind of *Citrus nobilis deliciosa.* It has been synthesized starting from 2,6-dihydroxy-ω-3,4-trimethoxyacetophenone.



(III)

Herbacetin (IV),[123] $C_{15}H_{10}O_7$ (3,5,7,8,4′-pentahydroxyflavone, pale yellow needles, m.p. 280-283°), is present as the glycoside herbacitrin, $C_{21}H_{20}O_{12}$ (yellow needles, m.p. 277-279°), in *Gossypium indicum* and *herbaceum.* A synthesis [124] from anisic acid and 2,4-dihydroxy-ω,3,6-trimethoxyacetophenone has been achieved. The difficulty of elucidating the constitution of herbacitrin, and incidentally of quercimeritrin also, by direct complete methylation has been overcome by simultaneously deacetylating and methylating acetyl derivatives. The results confirm that herbacitrin and quercimeritrin are 7-glucosides of herbacetin and quercetin, respectively.[124a]

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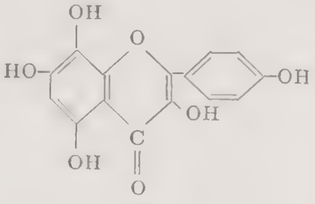
[120] Anderson, A. G. Perkin: *J. Chem. Soc.*, **1931,** 2624; Anderson: *Canadian J. Res.*, **7,** 285 (1932); **9,** 80 (1933); *cf.* also Badhwar, King, Venkataraman: *J. Chem. Soc.*, **1932,** 1107.

[121] Gulati, Vankataraman: *J. Chem. Soc.*, **1933,** 942, 1644.

[122] Nelson: *J. Am. Chem. Soc.*, **56,** 1392 (1934); Goldsworthy, Robinson: *J. Chem. Soc.*, **1937,** 46.

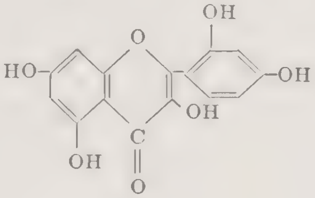
[123] Neelakantam, Seshadri, Rao: *Chem. Zentr.*, **1936,** I, 3518; Neelakantam, Seshadri: *Chem. Zentr.*, **1937,** I, 887; **1937,** II, 2184.

[124] Goldsworthy, Robinson: *J. Chem. Soc.*, **1938,** 56.



(IV)

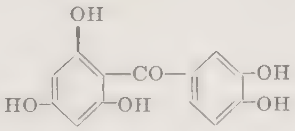
Morin,[125] (V), $C_{15}H_{10}O_7$ (3,5,7,2′,4′-pentahydroxyflavone, yellow needles, m.p. 290°) is the coloring principle of the wood of the American and Indian *Morus tinctoria* (dyer's mulberry). On fission with alkali, phloroglucinol and β-resorcylic acid were obtained and the compound was synthesized[126] from 2,4-dimethoxybenzaldehyde and phloracetophenone dimethyl ether.



(V)

With an extract of the compound on chromium-mordanted wool, the dyeings are fast to washing and fulling, but not to light. Morin is used in conjunction with alum, copper, or iron mordants to obtain olive-green shades on cotton, and pure morin is used in cotton printing (calico yellow).

Accompanying morin is maclurin (morin tannic acid), $C_{13}H_{10}O_6$ (pale-yellow crystals, m.p. 220-222°), which is 2,4,6,3′,4′-pentahydroxybenzophenone:



It is separated from morin by taking advantage of its solubility in hot water. Maclurin is degraded by alkali to phloroglucinol and protocatechuic acid, and has been synthesized[127] from protocatechuic nitrile and phloroglucinol.

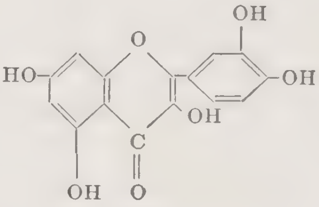
[124a] Suryaprakasa, Rao, Seshadri: *Proc. Indian Acad. Sci.*, **9A**, 365 (1940).

[125] Schultz: "Farbstofftabellen," 7 ed., I, p. 627, No. 1366.

[126] Earliest synthesis: v. Kostanecki, Lampe, Tambor: *Ber.*, **39**, 625 (1906).

[127] Hoesch, v. Zarzecki: *Ber.*, **50**, 462 (1917).

Quercetin,[128] (VI), $C_{15}H_{10}O_7$ (3,5,7,3′,4′-pentahydroxyflavone, yellow crystals, m.p. 316-317°), is one of the most widely distributed natural pigments. It occurs in many plants, among which may be mentioned the horse-chestnut, vine-leaves, hops, tea, sumach, red rose, onion skin and the North American dyer's oak, *Quercus tinctoria,* which is of value in the technical production of quercetin.



(VI)

Among glycosides[129] of quercetin may be mentioned: quercitrin, $C_{21}H_{20}O_{11}$ (yellow needles, m.p. 182-185°), the 3-rhamnoside occurring in the bark of *Quercus tinctoria;* isoquercitrin, $C_{21}H_{20}O_{12}$ (yellowish needles, m.p. 217-219°), the 3-glucoside, is found, *e.g.*, in maize,[130] in *Arctostaphylos uva ursi,* in *Vaccinium vitis idaea,*[131] and in *Trifolium* blossom[132]; in the cotton flower isoquercitrin is accompanied by the 7-glucoside quercimeritrin, $C_{21}H_{20}O_{12}$, also found in *Gossypium indicum*[133] and *Gossypium hirsutum*[134];* the 3-galactoside of quercetin,[135] $C_{21}H_{20}O_{12}$ (m.p. 236.5-237.5°), is present in Grimes Golden and Jonathan apples; incarnatrin, $C_{21}H_{20}O_{12}$ (yellow needles, decomposing at 242-245°) from *Trifolium incarnatum*[136] and a similar product (yellow needles, m.p. 228-229°) from *Ambrosia artemisifolia*[137] may be glycosides of quercetin; rutin[138] (sophorin, osyritrin, violaquercitrin, myrticolorin, globulariacitrin), $C_{27}H_{30}O_{16}$ (pale yellow needles, m.p. 180-190°), is the 3-rutinoside (rutinose contains rhamnose and glucose units) occurring in the flower buds of *Sophora japonica* (rutoside[139]), in common rue (*Ruta graveolens*), in the flower buds of *Capparis spinosa,* in *Eschscholtzia californica* Cham., in elderberry blossom[140] (*Sambucus canadensis*), in the leafy stalks of

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*This constitution for quercimeritrin has recently been conclusively proved.[134a]

[128] Schultz: "Farbstofftabellen," 7 ed., I, p. 628/9, No. 1367 and 1368.

[129] Position of sugar residues: Attree, A. G. Perkin: *J. Chem. Soc.*, **1927,** 234; glycosides: Zemplén, Csürös, Gerecs, Aczél: *Ber.*, **61,** 2486 (1928).

[130] Sando, Bartlett: *J. Biol. Chem.*, **54,** 629 (1922).

[131] A. G. Perkin: *Proc. Chem. Soc. London,* **14,** 104 (1898); Nakamura, Ohta, Hukuti: *J. Pharm. Soc. (Japan),* **55,** 198, 252 (1935).

[132] Hattori, Hasegawa, Hayashi: *Acta phytochimica (Japan),* **10,** 147 (1937).

[133] Neelakantam, Seshadri: *Chem. Zentr.*, **1937,** I, 887.

[134] Neelakantam, Rao, Seshadri: *Chem. Zentr.*, **1936,** I, 3518.

[134a] Suryaprakasa, Rao, Seshadri: *Proc. Indian Acad. Sci.*, **9A,** 365 (1939).

[135] Sando: *J. Biol. Chem.*, **117,** 45 (1937).

[136] Rogerson: *J. Chem. Soc.*, **97,** 1004 (1910).

[137] Heyl: *J. Am. Chem. Soc.*, **41,** 1285 (1919).

[138] Sando, Bartlett: *J. Biol. Chem.*, **41,** 495 (1920); Zemplén, Gerecs: *Ber.*, **68,** 1318 (1935).

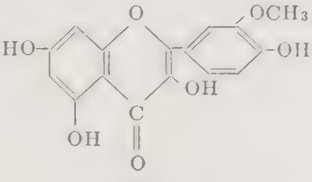
[139] Rabaté, Dussy: *Bull. soc. chim. biol.*, **20,** 467 (1938).

[140] Sando, Lloyd: *J. Biol. Chem.*, **58,** 737 (1924).

Umbellifere Bupleurum falcatum[141] and in the stalks of the tomato.[142] Querciturон,[143] $C_{21}H_{18}O_{13}$ (pale yellow needles, m.p. 192°), has been isolated from the leaves of etiolated beans; it is the chief yellow water-soluble pigment of the bean, and may be degraded to quercetin and glycuronic acid. Glycosides of quercetin, together with those of isoquercetin, luteolin, and other flavones, have also been found in *Equisetum arvense* L.[143a] Quercetin is broken down by fission with alkali into phloroglucinol and protocatechuic acid and has been obtained synthetically from veratraldehyde and the dimethyl ether of phloracetophenone.[144]

Quercitron extract[145] (quercitron is the bark of dyer's oak) finds application under the name flavin in dyeing and more especially in printing. Quercitron gives green-yellow shades compared with the orange-red tones of quercetin. Chinese avignon (yellow) berries are the buds of *Sophora japonica* mentioned above. The rutin contained in the berries undergoes no change on dyeing, and brown-yellow-orange shades are obtained on wool mordanted with aluminum. Quercetin is obtained from an alcoholic extract of quercitron.

Isorhamnetin, (VII), the 3′-methyl ether of quercetin, $C_{16}H_{12}O_7$ (yellow needles, m.p. 305°), has been found in "asbarg," the flowers and flower stems of *Delphinium zalil,* in senna leaves and in *Typha angustata.* It is isolated from "asbarg" by extraction with water. The isorhamnetin ester of potassium bisulfate, $C_{16}H_{11}O_{10}SK$ (m.p. 280°), occurs in *Persicaria Hydropiper* Opiz.[146]

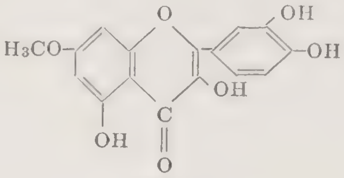


(VII)

Rhamnetin, the 7-methyl ether of quercetin, (VIII), $C_{16}H_{12}O_7$ (lemon-yellow powder), occurs as the 3-trirhamnoside xanthorhamnetin, $C_{34}H_{42}O_{20}$ (golden-yellow needles), in the berries of way- and buck-thorn (*Rhamnus* varieties) and in Persian berries, being most abundant in the berries[147] of *Rhamnus saxatilis, amygdalinus* and *oleides* (Turkey and Persia). The pigment comes into commerce as a buckthorn extract which is used in the form of a yellowish-brown chromium lake in cotton print-

[141] Rabaté: *Bull. soc. chim. biol.,* **12,** 974 (1930).
[142] Blount: *J. Chem. Soc.,* **1933,** 1528.
[143] Endres, Hüttel, Kaufmann: *Ann.,* **537,** 205 (1939).
[143a] Nakamura, Hakuti, *J. Pharm. Soc. Japan,* **60,** 449 (1940).
[144] cf. Allan, Robinson: *J. Chem. Soc.,* **1926,** 2334.
[145] Ullmann: "Encyclopädie der technischen Chemie," Vol. **5,** p. 142.
[146] Kawaguchi, Kim: *J. Pharm. Soc. (Japan),* **57,** 180 (1937).
[147] Ullmann, "Encyclopädie der technischen Chemie," Vol. **5,** p. 137.

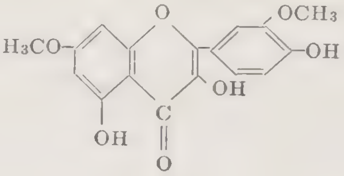
ing. On wool, orange and yellow shades are obtained using tin and aluminum mordants. Rhamnetin is isolated by extracting the berries with water.



(VIII)

A quercetin monomethyl ether, $C_{16}H_{12}O_7$ (yellow needles, acetyl derivative, m.p. 169-171°), of unknown constitution[148] occurs with ellagic acid in *Tamarix africana* and *Tamarix gallica*. It yields quercetin on heating with hydriodic acid.

Rhamnazin, (IX), the 3′-7-dimethyl ether of quercetin, $C_{14}H_{14}O_7$ (yellow needles, m.p. 214-215°), accompanies rhamnetin in buckthorn species. Rhamnazin dyes orange-yellow shades on aluminum mordants. Rhamnazin was found also in *Polygonum Hydropiper* L.[149]



(IX)

Scoparin.[150] This compound occurs in the broom *Spartium scoparium* together with spartein and also as scopariside in *Sarothamnus scoparius* Koch. It has the empirical formula $C_{22}H_{22}O_{11}$, and is regarded as a glycoside, as according to more recent investigations it yields rhamnose and quercetin. It forms yellow crystals (m.p. 228-230°) and yields phloroglucinol, acetovanillone, vanillic acid and protocatechuic acid on fusing with alkali. An alcoholic extract of broom flowers is a convenient source of the pigment.

Robinetin,[151] (X), $C_{15}H_{10}O_7$ (3,7,3′,4′,5′-pentahydroxyflavone, greenish-yellow needles decomposing at 325-330°), occurs in the wood of *Robinia pseudacacia* and in *Gleditschia monosperma*. Fission of the pentamethyl derivative yielded gallic acid trimethyl ether, and the dimethyl ether of fisetol and synthesis was effected from ω-methoxyresacetophenone and trimethylgallic acid with subsequent demethylation. Robinetin dyes cotton a brown-orange shade with an aluminum mordant. The pigment is isolated from an alcoholic extract of the ground wood of *Robinia pseudacacia.*

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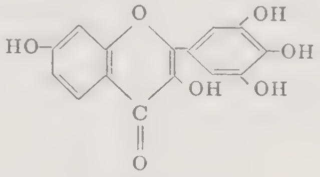
[148] A. G. Perkin, Wood: *J. Chem. Soc.*, **73**, 374 (1898).

[149] Krynska: *Chem. Zentr.*, **1935**, II, 2696

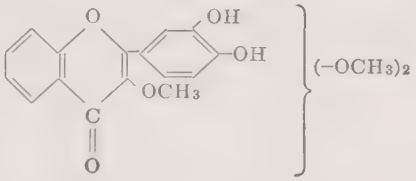
[150] Stenhouse: *Ann.*, **78**, 15 (1851); the formula $C_{21}H_{22}O_{10}$ is proposed; Hlasiwetz: *Ann.*, **138**, 190 (1866); Goldschmiedt, v. Hemmelmayr: *Monatsh.*, **14**, 202 (1893); **15**, 316 (1894); A. G. Perkin: *Proc. Chem. Soc. London*, **15**, 123 (1899); *J. Chem. Soc.*, **73**, 1030 (note) (1898); **77**, 423 (1900); Herzig, Tiring: *Monatsh.*, **39**, 253 (1918); Mascré, Paris: *Compt. rend.*, **204**, 1581 (1937); *Bull. Sci. Pharmacol.*, **44**, 401 (1937).

[151] Schmid, Pietsch: *Monatsh.*, **57**, 305 (1931); Schmid, Tadros: *Ber.*, **65**, 1689 (1932); Brass, Kranz: "Cellulose Chemie," **12**, 173 (1931); *Naturwiss.*, **20**, 672 (1932); *Ann.*, **499**, 175 (1932); *Ber.*, **65**, 1867 (1937); Charlesworth, Robinson: *J. Chem. Soc.*, **1933**, 268; Gulati, Venkataraman: *J. Pract. Chem. (2)*, **137**, 294 (1933).

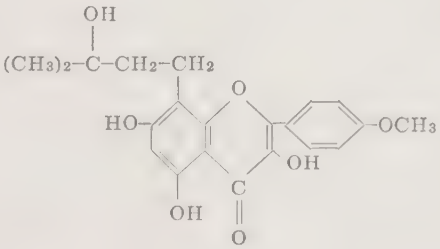


(X)

Amarbelin,[152] $C_{18}H_{16}O_7$ (brown-yellow needles, m.p. 234°) has been isolated from the seeds of *Cuscuta reflexa* Roxb. Fusion with potash gave protocatechuic acid and a phenol, and the following constitutional formula is provisionally assigned:



Icariin,[153] $C_{33}H_{42}O_{16}$ (yellow needles, m.p. 231.5°), a compound found in one of the wild *Berberidaceae, Epimedium macranthum* Morr et Decne ("Icariso"), may be resolved into rhamnose and a second compound icariside, $C_{27}H_{32}O_{12}$; the latter forms yellow needles (m.p. 256°) and is itself hydrolyzed to glucose and icaritin (XI), $C_{21}H_{22}O_7$ (yellow needles, m.p. 239.5°). Icaritin quickly loses one molecule of water, passing into anhydroicaritin, $C_{21}H_{20}O_6$ (yellow needles, m.p. 142-143°). The trimethyl esters of both icaritin and anhydroicaritin have been synthe-



(XI)

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[152] Agarwal: *J. Indian Chem. Soc.*, **13**, 531 (1936).

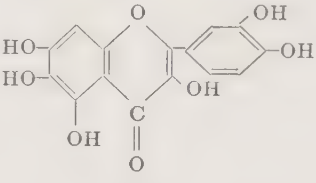
[153] Akai: *J. Pharm. Soc. (Japan)*, **55**, 112 (1935); Akai, Matsukawa: *Ibid.*, **55**, 129 (1935); Akai, Nakazawa: *Ibid.*, **55**, 135 (1935).

sized.[154] Des-*O*-methylicariin,[155] $C_{32}H_{40}O_{16}$ (yellow needles, m.p. 235-237°), is a further glycoside found in nature. Icariin is used as an aphrodisiac.

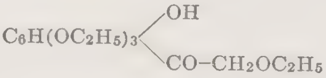
Tambutin, a pigment from *Xanthoxylum acanthopodium,* forms deep yellow plates, m.p. 205°. It is believed to be 5:7-dihydroxy-3:8:4′- or -6:8:4′-trimethoxyflavone.[155a]

Hexahydroxyflavones

Quercetagetin, (I), $C_{15}H_{10}O_8$ (3,5,6,7,3′,4′-hexahydroxyflavone, yellow needles, m.p. 318-320°, with decomposition), occurs in the flowers of *Tagetes patula* and *erecta.*[156] The formula given is based on fission of the hexaethyl ether into diethylprotocatechuic acid and quercetagol tetraethyl ether,

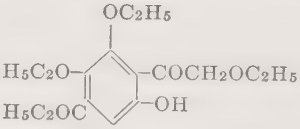


(I)



$$C_6H(OC_2H_5)_3\left\langle\begin{array}{l}OH\\CO{-}CH_2OC_2H_5\end{array}\right.$$

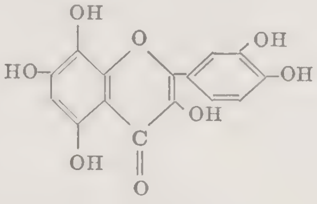
and synthesis [157] from veratric acid and 2,6-dihydroxy-ω,3,4-trimethoxyacetophenone. Quercetagetin is obtained from an alcoholic extract of the blooms.



Gossypetin, (II), $C_{15}H_{10}O_8$ (3,5,7,8,3′,4′-hexahydroxyflavone, yellow needles, m.p. 310-314°), is found in the form of its 7-glucoside [158] gossypitrin, $C_{21}H_{20}O_{13}$ (orange-yellow needles,.m.p. 250-252°), together with quercimeritrin in cotton flower.[159] Gossypin,[160] $C_{28}H_{24}O_{18}$ (golden-yellow needles, m.p. 230-231°) is another glycoside of gossypetin found in *Gossypium indicum.* Fission of the hexamethyl ether of gossypetin yielded dimethylprotocatechuic acid and a phenol, gossipitol tetramethyl ether;

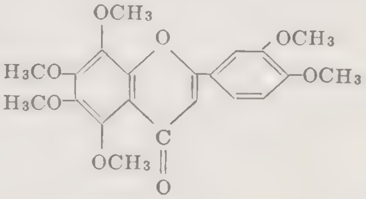
[154] Akai, Nakazawa: *Ibid.,* 55, 153 (1935).
[155] Akai, Imaida, Matsukawa: *Ibid.,* 55, 214 (1935).
[155a] Bose, P. K., and Bose, S.: *J. Indian Chem. Soc.,* 16, 183 (1939).
[156] Mahal: *J. Indian Chem. Soc.,* 15, 87 (1938).
[157] Baker, Nodzu, Robinson: *J. Chem. Soc.,* 1929, 74.
[158] Neelakantam, Seshadri: *Chem. Zentr.,* 1938, I, 2370; Rao, Seshadri: *Ibid.,* 1939, II. 1080.
[159] Neelakantam, Seshadri: *Ibid.,* 1936, I, 3518.
[160] Neelakantam, Seshadri: *Ibid.,* 1937, I, 887.

the structure deduced was synthesized[161] from veratric acid and 2,4,-dihydroxy-ω,3,6-trimethoxyacetophenone. Gossypetin is prepared from an alcoholic extract of the flowers of the cotton plant.

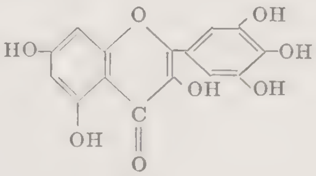


(II)

Nobiletin,[162] $C_{21}H_{22}O_8$ (yellow crystals, m.p. 134°), a compound present in the Chinese drug Chen pi (*Citrus nobilis* Lour.), contains 6 methoxyl groups and is possibly 5,6,7,8,3′,4′-hexamethoxyflavone:



Myricetin,[163] (III), $C_{15}H_{10}O_8$ (3,5,7,3,4′,5′-hexahydroxyflavone, pale-yellow needles, m.p. 357-360°), occurs in the leaves and bark of *Myrica nagi* and in various *Rhus* species as the 3-rhamnoside myricitrin, $C_{21}H_{22}O_{13}$ (yellow leaflets, m.p. 199-200°). It has been synthesized from ω-methoxyphloracetophenone and the trimethyl ether of gallic acid, followed by hydrolysis of the ether groups. Myricetin is prepared from an aqueous extract of the bark of *Myrica nagi*.



(III)

Cannabiscetin,[164] $C_{15}H_{10}O_8$, a pentahydroxyflavonol, occurs as a glycoside, cannabiscitrin, $C_{21}H_{20}O_{13}$, in flowers of Deccan hemp, (*Hibiscus cannabinus*). The sugar residue is glucose.

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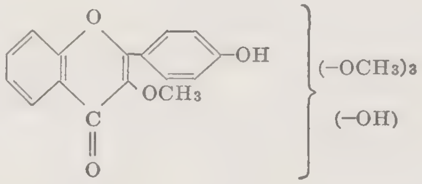
[161] Baker, Nodzu, Robinson: *J. Chem. Soc.*, 1929, 74.

[162] Tseng: *Ibid.*, 1938, 1003; Robinson, Tseng: *Ibid.*, 1938, 1004.

[163] A. G. Perkin: *Ibid.*, 81, 208 (1902); Kalff, Robinson: *Ibid.*, 127, 181 (1925); Nierenstein: *Ber.*, 61, 361 (1928); Hattori, Hayashi: *Acta phytochimica (Japan)*, 5, 213 (1931).

[164] Neelakantan, Seshadri: *Chem. Zentr.*, 1938, II, 3091.

Calycopterin,[165] (=Thapsin), $C_{19}H_{18}O_8$ (lemon-yellow prisms, m.p. 224°) has been isolated from the shrub *Calycopteris floribunda* and from a digitalis drug, probably *Digitalis Thapsi* L. It is a dihydroxytetramethoxyflavone, the fully methylated compound being degraded to *p*-methoxybenzoic acid and 2-hydroxy-3,4,5,6,ω-pentamethoxyacetophenone. As one hydroxyl group is located in the benzene ring and it is known that the 3-position bears a methoxy group, the following partial formula is suggested:



Gardenin,[166] Decamalee or Dicamali gum, the resinous secretion of *Gardenia lucida* (India) contains a pigment which, according to recent research, has the formula $C_{21}H_{22}O_9$. Forming yellow needles (m.p. 161-162°) it is regarded as 5-hydroxy-3,6- or 7,8-3′,4′,5′-hexamethoxyflavone, and on fission yields trimethylgallic acid.

Erianthin, $C_{20}H_{20}O_9$, yellow needles, m.p. 154°, occurs in the flowers of Blumea eriantha. Its formulation as 5:7-dihydroxy-3:6:8:3′:4′-pentamethoxyflavone is supported by the following considerations: Veratric acid is formed on alkaline degradation; one hydroxyl group in the 5-position is likely, from the formation of a monomethyl ether; the other hydroxyl group must be in the same ring (a hydroxyl in the 3-position is unlikely from the stability of erianthin toward oxygen in alkaline solution) and in the *m*-position to the first.[166a]

Isoflavones

Daidzein,[167] (I), $C_{15}H_{10}O_4$ (7,4′-dihydroxyisoflavone, pale-yellow prisms, m.p. 315-320°), occurs as its 7-glucoside, daidzin, in Soja hispida (Japanese "daidzu"), $C_{21}H_{20}O_9$ (colorless prisms, m.p. 235°). Daizein breaks down on fission with alkali into formic acid and 2,4-dihydroxyphenyl-4′-hydroxybenzyl ketone, and it has been synthesized [168] from this ketone and formic ester.

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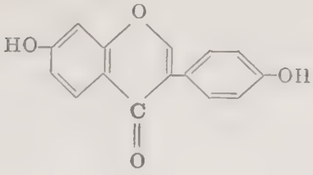
[165] Ratnagiriswaran, Sehra, Venkataraman: *Biochemical J.*, **28**, 1964 (1934); W. Karrer: *Helv. Chim. Acta*, **17**, 1560 (1934); W. Karrer, Venkataraman: *Nature*, **135**, 878 (1935).

[166] Stenhouse, Groves [*Ann.*, **200**, 311 (1880)] give the literature and earlier work of Dymock and Flückiger; Bose: *Chem. Zentr.*, **1937**, I, 3810; Bose, Nath: *J. Indian Chem. Soc.*, **15**, 139 (1938).

[166a] Bose, Dutt: *J. Indian Chem.. Soc.*, **17**, 45 (1940).

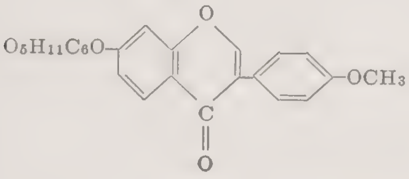
[167] Walz: *Ann.*, **489**, 118 (1931).

[168] Wessely, Kornfeld, Lechner: *Ber.*, **66**, 685 (1933); cf. Baker, Robinson, Simpson: *J. Chem. Soc.*, **1933**, 274, where the synthesis starts from an isoflavone; Mahal, Rai, Venkataraman: *J. Chem. Soc.*, **1934**, 1769.

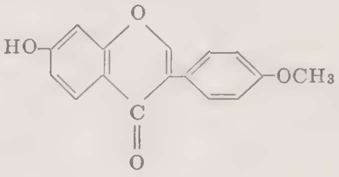


(I)

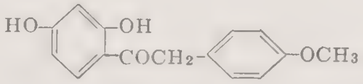
Daidzein is prepared from a methanol extract of soybeans. The plant *Ononis spinosa* contains a glycoside ononin[169] derived chiefly from the 4-methyl ether of daidzin, the contaminant having not yet been obtained in a state of purity. Ononin breaks down on hydrolysis with acid into form-onetin, and then with boiling baryta water into ononetin:



Ononin



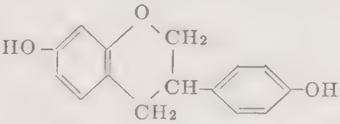
Form-onetin



Ononetin

Form-onetin[170] was prepared from 2,4-dihydroxyphenyl-4′-methoxybenzyl ketone and formic ester; and onospin obtained directly from ononin with baryta water and consisting to a predominating extent of 2-hydroxy-4-*d*-glucosidoxyphenyl-4-methoxybenzyl ketone, has been likewise synthesized.[171]

Equol,[172] $C_{15}H_{14}O_3$ (yellow needles, m.p. 189-190.5°), isolated from the urine of mares and stallions, is 7,4′-dihydroxyflavane:



It has been obtained from diadzein by hydrogenation.

Genistein,[173] (II), (prunetol), $C_{15}H_{10}O_5$ (5,7,4′-trihydroxyisoflavone, colorless needles, m.p. 291-293°), is contained in dyer's broom (*Genista*

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[169] Baker, Eastwood: *Ibid.*, **1929,** 2897; Wessely, Lechner: *Monatsh.*, **57,** 395 (1931).

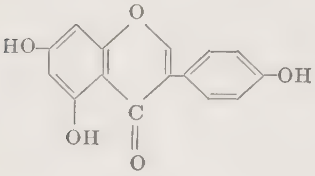
[170] Wessely, Kornfeld, Lechner: *Ber.*, **66,** 685 (1933); Wessely, Lechner, Dinjaški: *Monatsh.*, 63, 201 (1933).

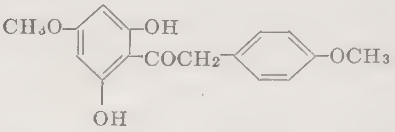
[171] Baker, Eastwood: *J. Chem. Soc.*, **1929,** 2897; Wessely, Lechner: *Monatsh.*, **57,** 395 (1931).

[172] Marrian, Haslewood: *Biochem. J.*, **26,** 1227 (1932); Marrian, Beall: *Ibid.*, **29,** 1586 (1935); Wessely, Hirschel, Schlögl-Petziwal: *Monatsh.*, **71,** 215 (1938).

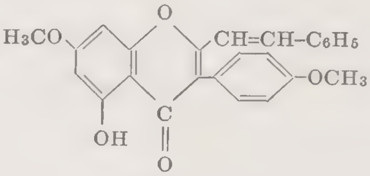
[173] Baker, Robinson: *J. Chem. Soc.*, **127,** 1981 (1925); **1926,** 2713; **1928,** 3115; Identity with prunetol from Prunus serotina: Baker, Robinson: *Ibid.*, **1926,** 2713; Walz: *Ann.*, **489,** 118 (1931).

tinctoria), together with luteolin, and in soybeans (*Soja hispida*) as the 7-glucoside genistin, $C_{21}H_{20}O_{10}$ (?) (yellow leaflets, m.p. 254-256°). Alkali fission afforded formic and *p*-hydroxyphenylacetic acids and phloroglucinol, and the compound has been synthesized from


(II)



and cinnamic anhydride through the intermediate 5-hydroxy-7,4′-dimethoxy-2-styrylisoflavone (III):


(III)

Genistein is isolated from an aqueous extract of dyer's broom after separating luteolin; soybeans may also be used as a source.

A glycoside, sophoricoside,[174] $C_{21}H_{20}O_{11}$ (prisms, m.p. 297.5°), is known to occur in *Sophora japonica* L. This contains both the glucose and genistein residues, and in spite of the different melting point reported is probably identical with genistin.

Prunetin,[175] $C_{16}H_{12}O_5$ (colorless needles, m.p. 242°) is the 4- or 7-methyl ether of genistein, and occurs as the glycoside prunitrin, $C_{22}H_{14}O_{11}$, in the bark of a *Prunus* variety apparently related to *Prunus emarginata* and *Prunus avium;* it comes into commerce as a substitute for *Prunus serotina.* It is obtained by boiling an aqueous extract of the bark with hydrochloric acid.

Another so-called dihydroxymethoxyisoflavone glycoside D,[176] hydrolysis of which provides a dihydroxymethoxyisoflavone, $C_{16}H_{12}O_5$ (colorless needles, m.p. 310°), is found in *Soja hispida.* The isoflavone undergoes fission to formic acid and what is probably 2,3-dihydroxyphenyl-*x*,*x*′-hydromethoxybenzyl ketone. Still another isoflavone E possibly exists in the mother-liquor.

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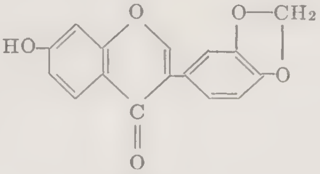
[174] Charaux, Rabaté: *J. pharm. chim. (8)*, **21**, 546 (1935); *(8)*, **22**, 32 (1935); *Bull. soc. chim. biol.*, **20**, 454 (1938).

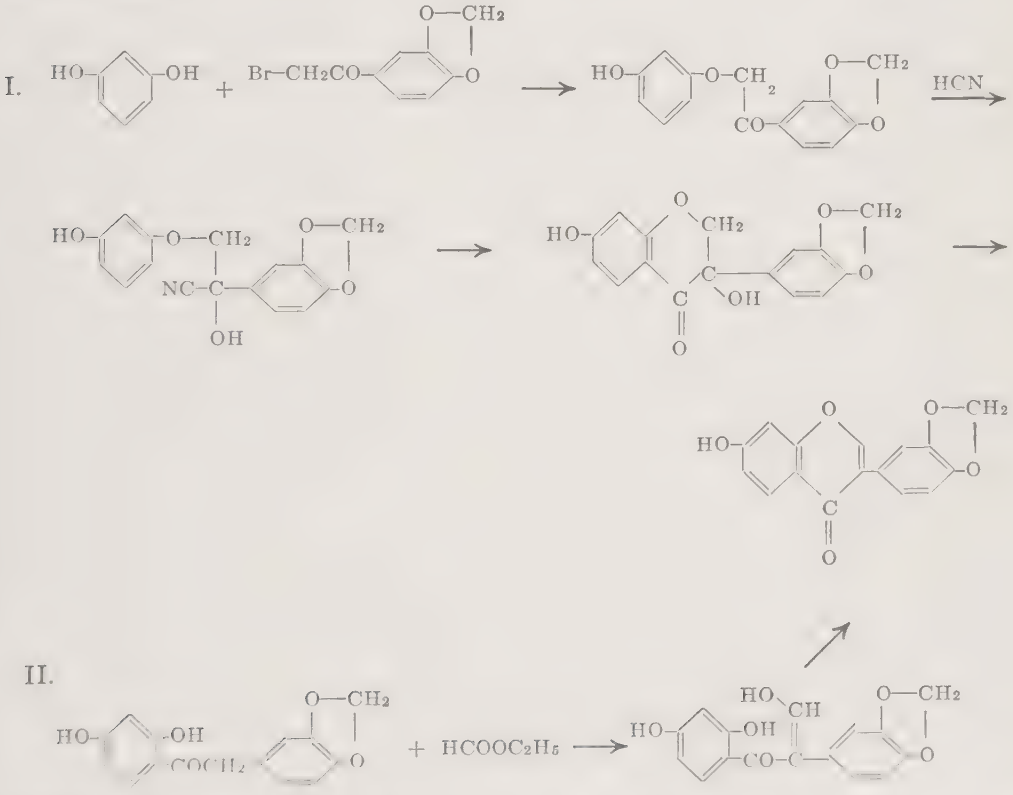
[175] Baker, Robinson: *J. Chem. Soc.*, **127**, 1981 (1925); **1926**, 2713; **1928**, 3115; Finnemore: *Pharm. J. (4)*, 31, 604 (1910); Baker: *J. Chem. Soc.*, **1928**, 1022.

[176] Walz: *Ann.*, **489**, 118 (1931).

Pseudobaptigenin,[177] (IV), $C_{16}H_{10}O_5$ (7-hydroxy-3′,4′-methylenedihydroxyflavone, colorless crystals, m.p. 298-299°), is contained as its 7-rhamnoside pseudobaptisin, $C_{28}H_{30}O_{14}$ (colorless crystals, m.p. 249-251°), in the root of *Baptisia tinctoria,* one of the North American *Papilionatae.* The constitution of pseudobaptigenin is established by the following facts: The phenolic hydroxyl groups and a carbonyl group may be detected; nitric acid affords styphnic acid and potassium permanganate degrades it to piperonylic acid; fission by alkali results in the production of formic acid and 2,4-dihydroxyphenyl-3′,4′-methylenedihydroxybenzyl ketone; finally pseudobaptigenin has been synthesized by the following two methods:



(IV)



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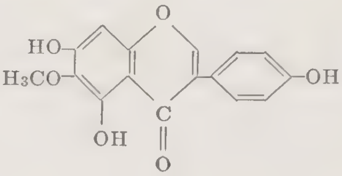
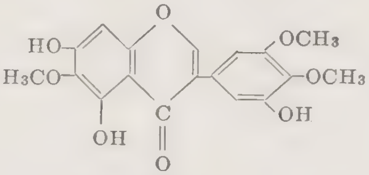
[177] Späth, Schmidt: *Monatsh.*, 53/54, 454 (1929); Späth, Lederer; *Ber.*, 63, 743 (1930); other syntheses: Mahal, Rai, Venkataraman: *J. Chem. Soc.*, 1934, 1769; Robinson, Simpson: *Ibid.*, 1937, 805.

Tectorigenin,[178] (V), $C_{16}H_{12}O_6$ (6-methoxy-5,7,4′-trihydroxyisoflavone, pale-yellow leaflets, m.p. 227°), is found as its 7-glucoside tectoridin $C_{22}H_{22}O_{11}$ (needles, m.p. 258°), in the rhizome of *Iris tectorum* Max., indigenous to Japan. Tectoridin[179] also occurs in the root-stock of the Chinese *Iridacea Belamcanda chinensis* (L) Leman. The so-called shekanin, isolated from the same plant by Wu,[180] who termed it *Pardanthus chinensis,* assigning the erroneous formula $C_{16}H_{16}O_8$, is identical with tectoridin. Tectorigenin on fission yields formic acid, *p*-hydroxyphenylacetic acid and iretol (1,3,5-trihydroxy-2-methoxybenzene). It is prepared from an alcoholic extract of the rhizomes.



(V)

Irigenin,[181] (VI), $C_{18}H_{16}O_8$ (5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavone, yellow plates, m.p. 185°), is present as its 7-glucoside iridin,[182] $C_{24}H_{26}O_{13}$ (colorless needles, m.p. 208°) in *Iris germanica, pallida* and *florentina.* Irigenin breaks down by alkali fission into formic acid, iretol (1,3,5-trihydroxy-2-methoxybenzene) and iridic acid (3-hydroxy-4,5-didimethoxyphenylacetic acid). Its tinctorial power is somewhat inferior to that of the flavones. Irigenin is conveniently prepared from commercial Florentine orris root.



(VI)

Catechin,[183] (VII), $C_{15}H_{14}O_6$, is known in *d*-, *l*- and *dl*-forms. The dextrorotatory modification is found in gambier, an extract of the leaves and branches of the Malayan liana *Uncaria gambir.* Two asymmetric carbon atoms are responsible for the optical isomerides which also include *d*-, *l*- and *dl*-epicatechin; *l*-epicatechin occurs in the wood of a number of Indian acacias, the dried sap being termed catechin. Only *d*-catechin and *l*-epicatechin appear to exist in nature.

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[178] Shibata: *J. Pharm. Soc. (Japan),* 1927, 61; Asahina, Shibata, Ogawa: *Ibid.,* 48, 150 (1928).

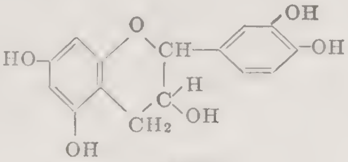
[179] Mannich, Schumann, Lin: *Arch. Pharm.,* 275, 317 (1937).

[180] Wu: *Chem. Zentr.,* 1936, I, 4012.

[181] Baker: *J. Chem. Soc.,* 1928, 1022; review of the earlier literature, Baker, Robinson: *Ibid.,* 1929, 152.

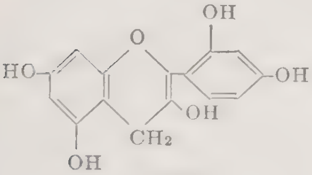
[182] Shinoda, Sato: *J. Pharm. Soc. (Japan),* 52, 139 (1932).

[183] Exhaustive discussion: Freudenberg in Klein, "Handbuch der Pflanzenanalyse," III, 2, p. 392: Review: Mason: *J. Soc. Chem. Ind.,* 1928, T, 269.

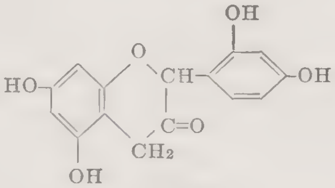


(VII)

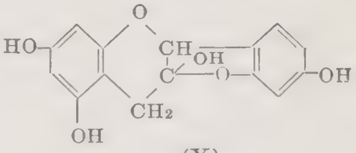
Cyanomaclurin[184] accompanies morin (from which it is separated by the different solubilities of the lead salts) in *Atrocarpus integrifolia.* It forms colorless prisms which darken at 290° without melting. Fusion with potash yields cresorcin and cresorcylic acid; the formula $C_{15}H_{12}O_6$ and constitution (VIII) were suggested by Perkin. Constitutions more recently proposed, however, have had to be reconciled with the facts that cyanomaclurin is optically active[185] and that Perkin's supposed penta-acetyl and -benzoyl derivatives are in reality tetra-substituted compounds. Formulas (IX) or (X) are therefore regarded as more probably correct:



(VIII)



(IX)



(X)

Soybean is known to contain at least four isoflavones, though their precise constitutions are not known with certainty:

Tatoin, $C_{16}H_{12}O_4$, needles, m.p. 318°, is tentatively formulated as 8-methyl-5:4′-dihydroxyisoflavone.

Methylgenistein, $C_{16}H_{12}O_5$, faintly yellow needles, m.p. 255°, the alkaline degradation of which indicates the structure 8-methyl-5:7:4′-trihydroxyisoflavone.

Methylisogenistin, $C_{22}H_{22}O_{10}$, is a glycoside. It is hydrolyzed to glucose and an aglucone, $C_{16}H_{12}O_5$, m.p. 301-302°. The aglucone is possibly 8-methyl-5:7:2′-trihydroxyisoflavone.

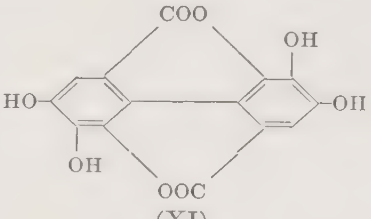
Isogenistin, $C_{21}H_{20}O_{10}$, is also a glycoside, giving glucose and an aglucone, $C_{15}H_{10}O_5$, m.p. 302°, on hydrolysis. The aglucone, it is suggested, is possibly 5:7:2′-trihydroxyisoflavone.[185a]

[184] A. G. Perkin, Cope: *J. Chem. Soc.*, **67**, 937 (1895); A. G. Perkin: *Ibid.*, **87**, 715 (1905); Freudenberg: *Ber.*, **53**, 1416 (1920); Pratt, Robinson: *J. Chem. Soc.*, **127**, 1128 (1925); Bhalla, Ray: *Ibid.*, **1933**, 288; Charlesworth, Chavan, Robinson: *Ibid.*, **1933**, 370; Mitter, Saha: *J. Indian Chem. Soc.*, **11**, 257 (1934); Mitter, Maitra: *Ibid.*, **13**, 236 (1936).

[185] Appel, Robinson: *J. Chem. Soc.*, **1935**, 752.

[185a] Okano, Beppu: *Bull. Agr. Chem. Japan*, **15**, 110 (1939).

Ellagic acid [186] is widely distributed in the plant kingdom, particularly in association with gallic acid or its derivatives; thus it is found in gall-apples, in the bark and wood of various oaks, in "dividivi" pods in the chestnut, etc. where it is probably bound in glycosidic form. The pigment, which has the constitution, $C_{14}H_6O_8$, (XI), forms a yellowish crystalline powder which sublimes without melting.



(XI)

Ellagic acid is obtained by extracting dividivi and other tanning agents [187] with water and boiling the solution with dilute sulfuric acid; it may also be obtained by oxidizing gallic acid with potassium persulfate and sulfuric acid in acetic solution. Ellagic acid dyes chromium-mordanted wool yellow, and was formerly obtainable in commerce as alizarin yellow.

Pigments of Flavone Character but of Incompletely Established Constitution

Vitexin and Homovitexin.[189] These two pigments occur as glycosides in the wood of *Vitex littoralis,* the puriri tree of New Zealand. Vitexin was earlier assumed to possess the formula $C_{15}H_{14}O_7$; this was later modified to $C_{21}H_{20}O_{10}$ on the basis of a supposed relationship to apigenin, *i.e.*, vitexin was regarded as a very stable glycoside of apigenin, but further work by Barger,[190] including determinations of the molecular weight, favor a return to the simpler formula. Barger found a substance which he termed saponarin, $C_{21}H_{24}O_{12}$, in the sap of the epidermal cells of the leaves of *Saponaria officinalis* (soap-wort); saponification of saponarin then yielded glucose, an amorphous material (saponaretin, see below under homovitexin) and vitexin. The vitexin obtained from these two sources has a melting point of 260° (Barger) or 264-265° (Perkin) and forms yellow prisms or needles; its (probably penta-) acetyl derivative crystallizes in needles (m.p. 257-258°). Vitexin dissolves in alkali to a pale-yellow solution.

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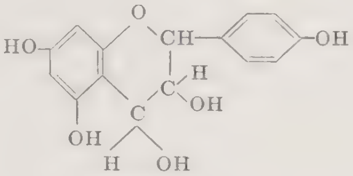
[186] Literature: Schultz: "Farbstofftabelle," 7 ed., p. 497, No. 1140; also Shinoda, Kun: *J. Pharm. Soc. (Japan)*, **51,** 50 (1931); Ullmann: "Encyclopädie der technischen Chemie," 2 ed., Vol. **5,** p. 131.

[187] Zetzsche, Graef: *Helv. Chim. Acta,* **14,** 240 (1931).

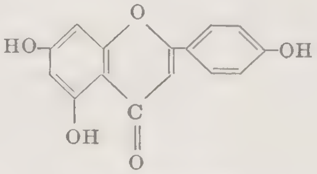
[189] A. G. Perkin: *J. Chem. Soc.,* **73,** 1019 (1898); *Proc. Chem. Soc. London,* **16,** 44 (1900); *J. Chem. Soc.,* **77,** 422 (1900).

[190] Barger: *Ber.,* **35,** 1296 (1902); *Chem. News,* **90,** 183 (1904); *J. Chem. Soc.,* **89,** 1210 (1906); the earlier literature is given here; *Chem. News,* **104,** 139 (1911).

On fusing with alkali, vitexin yields phloroglucinol, *p*-hydroxybenzoic acid and acetic acid, and *p*-hydroxyacetophenone arises by boiling with caustic potash. Boiling dilute nitric acid yields 1-hydroxy-3,5-dinitrobenzene-4-carboxylic acid, picric acid and a compound $C_{15}H_6O_5(NO_2)_4$ (m.p. 239-241°) which is probably identical with tetranitroapigenin. From the formula, degradation and behavior on nitration, Barger concluded that vitexin, which differs from apigenin in its empirical formula in that it contains the elements of two more molecules of water, contains two more hydroxyl groups than apigenin in the pyrone ring; thus the formation of *p*-hydroxybenzoic acid and tetranitroapigenin becomes explicable:



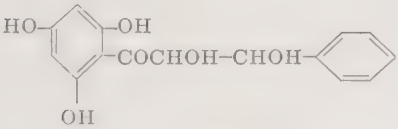
Vitexin(?)



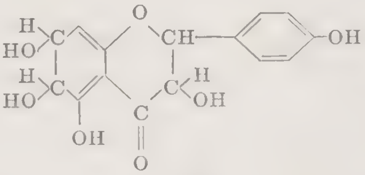
Apigenin

Attempts to dehydrate vitexin by orthodox methods and so convert it into apigenin failed; sublimation with zinc dust however gave a compound, $C_{15}H_{12}O_6$, the acetyl derivative of which appeared to be identical with that of apigenin.[190a]

The formulation of vitexin as a chalkone derivative (I) might merit consideration if not contradicted by the fact that such a formula contains 6 hydroxyl groups, whereas vitexin takes up only 5 acetyl groups; A. G. Perkin therefore suggested privately to Barger a formula containing a reduced phloroglucinol nucleus (II).



(I)



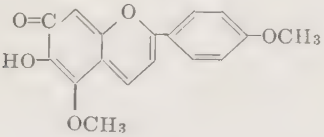
(II)

Homovitexin possesses the formula $C_{16}H_{16}O_7$ or $C_{18}H_{18}O_8$ and forms yellow needles (m.p. 245-246°). It contains no methoxyl groups, yields phloroglucinol and *p*-hydroxybenzoic acid on fusion with alkali, and is possibly identical with saponaretin.

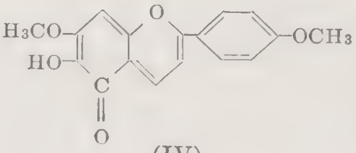
Both vitexin and homovitexin dye mordanted wool and cotton a pure, but weak, yellow somewhat similar to that obtained with apigenin. To isolate the pigments the ground wood is boiled for 8 hours with 10 volumes of water and the extract evaporated. The residue is then warmed with alcohol, filtered, and the filtrate once more evaporated. The orange resin so obtained contains the pigments still in the form of glycosides, which are hydrolyzed by dilute hydrochloric acid at room temperature. The semi-solid mass which is thus precipitated is treated with boiling alcohol; a yellow crystalline powder remains and is washed with alcohol until the filtrate remains colorless. This product, after further purification by conversion into its acetyl derivative and regeneration of the pure compound, affords pure vitexin. From the mother-liquor above homovitexin is obtained and purified by crystallization from alcohol.

[190a] Péteri, *J. Chem. Soc.*, 1939, 1635.

Chica red.[191] This somewhat rare pigment, also termed crajura or carajura, appears in the leaves of the Brazilian tree *Bignonia chika*. Chica red consists of two chemical entities, carajurin and carajurone, of which the former forms ruby red needles (m.p. 205-207°), $C_{17}H_{14}O_5$, yields oxonium salts and passes on demethylation into carajuretin iodide, a tetrahydroxyflavylium salt which has been synthesized from *p*-acetylanisole and antiarol aldehyde (2,3,4-trimethoxy-6-hydroxybenzaldehyde. Carajuretin is thus identical with scutellareinidin, and carajurin itself is in all probability represented by formula (III). Formula (IV) would seem to be less likely as such a structure would be expected to exhibit a blue color:



(III)



(IV)

Carajurone forms a scarlet-red microcrystalline powder (m.p. 183-186°) having the formula $C_{15}H_9O_5(OCH_3)$. On distillation an odor of *p*-hydroxybenzaldehyde was noticed and *p*-acetylanisole was obtained by alkaline fission. Carajurin and carajurone dye aluminum-mordanted wool and cotton a dull red-brown shade, and carajuretin gives dull orange-brown dyeings.

The pigment is used by the Indians of Rio Meta and Orinoco to color the skin. The leaves are extracted with water and the extract treated with a powder from the bark of "aryane," whereby the precipitation of the coloring matter is accelerated, probably following enzymic fission of the glycoside. The red cake containing 4 per cent of pigment comes into commerce under the names *carneru* or *vermillion americanum*. Its purification is somewhat arduous.

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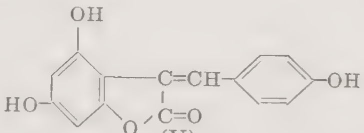
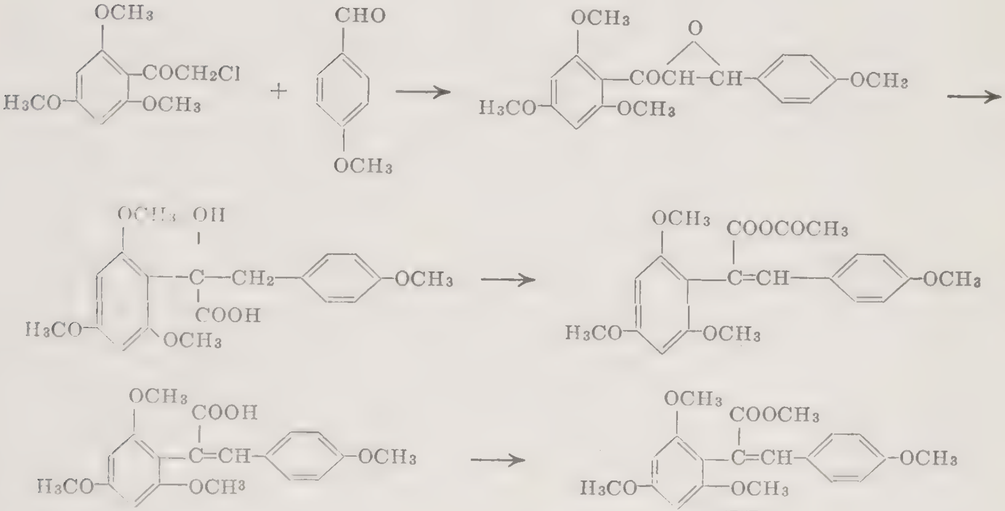
[191] Chapman, A. G. Perkin, Robinson: *J. Chem. Soc.*, 1927, 3015; earlier work, A. G. Perkin: *Proc. Chem. Soc. (London)*, 30, 212 (1914); Holmes: *Pharm. J.*, 12, 595 (1901).

Fukugetin[192] is a pigment occurring in the bark of *Garcinia spicata* and *Xanthocymus ovalifolia.* Although varying empirical molecular formulas have been proposed, that of Murakami,[193] $C_{24}H_{16}O_9$, would appear to meet with general acceptance. The pigment forms yellow crystals (m.p. 288-290°), dissolves in alkalies and in concentrated sulfuric acid to yellow solutions, and contains no methoxyl groups. According to Shinoda[194] fukugetin yields, on treating with 50-per cent caustic potash solution, 3,4-dihydroxyacetophenone, garcinol and fukugetin. To garcinol Murakami assigns the formula $C_{15}H_{10}O_5$ and the constitution (V), which expresses its readiness to take up two atoms of hydrogen by catalytic reduction and which has been verified synthetically:


(V)


(VI)

The ester (VI) was identical with pentamethyl garcinol hydrate obtained by the interaction of garcinol, dimethyl sulfate, and potassium hydroxide.

Fukugetin itself gives a pentamethyl ether, $C_{29}H_{26}O_9$ (m.p. 203°), and contains neither carboxyl nor lactone groupings. It may be concluded that the phloroglucinol and *p*-hydroxyphenyl groups must be linked in a manner different from that in garcinol, and indeed the formation of 3,4-dihydroxyacetophenone, phloroglucinol and protocatechuic acid on fission with caustic potash would indicate the presence of a flavone nucleus. Constitution (VII) has been suggested for fukegetin[195]:

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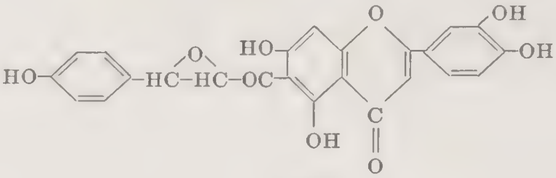
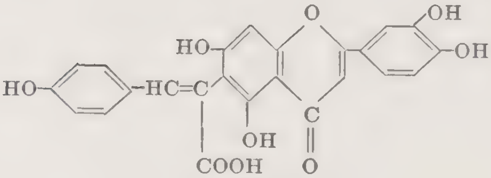
[192] A. G. Perkin, Phipps: *J. Chem. Soc.*, **85**, 56 (1904); Shinoda: *J. Pharm. Soc. (Japan)*, **1926**, 69; **1927**, 35; **52**, 167 (1932); Ito: *Chem. Zentr.*, **1908**, I, 1842 (nitro-dyestuffs).

[193] Murakami: *Proc. Imp. Acad. Tokyo*, **8**, 500 (1932); *Chem. Zentr.*, **1934**, II, 2394; Murakami, Irie: *Proc. Imp. Acad. Tokyo*, **10**, 568 (1934); *Chem. Zentr.*, **1935**, II, 233.

[194] Shinoda: *J. Pharm. Soc. (Japan)*, **53**, 167 (1933).



(VII)



(VIII)

whereas Shinoda's fukegenetin (isofukugetin of Murakami) is regarded by the latter worker as being an isomeride with constitution (VIII).

The coloring matter is extracted from the bark by water, the solution boiled with hydrochloric acid to bring about hydrolysis of the glycoside, and free pigment is taken up in alcohol and further purified. Fukugetin is used in Japan under the name *fukugi* to produce yellow dyeings. Garcinin,[196] which is said to occur in fukugi-bark, is regarded by Murakami[197] as impure fukugetin.

Rottlerin.[198] The pigment is present in the drug kamala, a product of the shrub *Mallotus phillipensis,* termed after a missionary *Rottlera tinctoria;* the plant belongs to the family of *Euphorbiaceae* (*Crotoceae*) and is native to Ceylon, India, China and Australia. Kamala itself is an orange-red powder which occurs as a glandular excrescence on the fruits and is gathered by shaking the ripe capsules in bags until the powder separates. It has for long been used for dyeing silk an orange color and also as an anthelmintic.

Rottlerin[199] probably has the empirical molecular formula $C_{30}H_{28}O_8$ (Narang, Ray, Roy: $C_{31}H_{30}O_8$) and crystallizes in delicate salmon-

[195] Murakami, Irie: *Bull. Chem. Soc. (Japan),* **11,** 288 (1936).

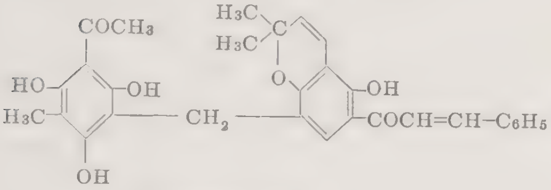
[196] Shinoda: *J. Pharm. Soc. (Japan),* **1927,** 33, 35 (proof that garcinin is not identical with 3-methylluteolin; synthetic attempts: Robinson, Shah: *J. Chem. Soc.,* **1933,** 610; Shinoda: *J. Pharm. Soc. (Japan),* **52,** 167 (1932); Murakami: *Proc. Imp. Acad. (Tokyo),* **8,** 500 (1932); *Chem. Zentr.,* **1934,** II, 2394.

[197] Murakami, Irie: *Proc. Imp. Acad. (Tokyo),* **10,** 568 (1934); *Chem. Zentr.,* **1935,** II, 233.

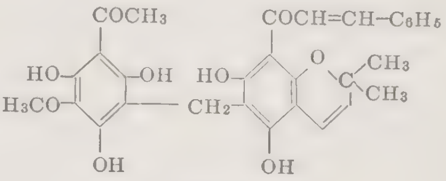
[198] Termed mallotoxin by Perkin.

[199] A. G. Perkin, W. H. Perkin, Jr.: *Ber.,* **19,** 3109 (1886); Jawein: *Ber.,* **20,** 182 (1887); A. G. Perkin: *J. Chem. Soc.,* **63,** 975 (1893); **67,** 230 (1895); Bartollotti: *Gaz. chim. ital.,* **24,** I, 4 (1894); **24,** II, 480 (1894); Telle: *Arch. Pharm.,* **244,** 441 (1906); earlier literature: Thoms: *Arch. Pharm.,* **244,** 640 (1906); Herrmann: *Arch. Pharm.,* **245,** 572 (1907); Dutt: *J. Chem. Soc.,* **127,** 2044 (1925); Dutt, Goswami: *J. Indian Chem. Soc.,* **5,** 21 (1928); A. Hoffmann, Fari: *Arch. Pharm.,* **271,** 97 (1933); Brockmann, Maier: *Naturwiss.,* **25,** 460 (1937); **26,** 14 (1938); McGookin, Reed, Robertson: *J. Chem. Soc.,* **1937,** 748; Narang, Ray, Roy: *Ibid.,* **1937,** 1862; *J. Indian Chem. Soc.,* **15,** 393 (1938); McGookin, Percival, Robertson: *J. Chem. Soc.,* **1938,** 309; Brockmann, Maier: *Ann.,* **535,** 149 (1938); Pharmacological action of rottlerin: Nagamachi: *Chem. Zentr.,* **1923,** II, 270. Absorption spectrum: Morton, Sawires: *J. Chem. Soc.,* **1940,** 1052. Optical activity: Ray: *Curr. Sci.,* **9,** 80 (1940).

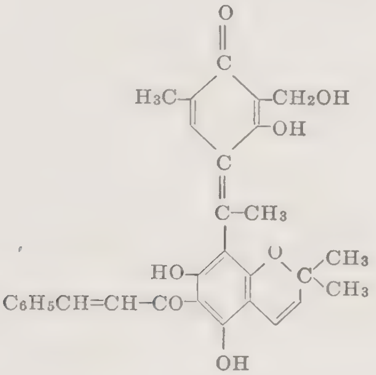
colored needles or plates (m.p. 201-202°). A considerable number of workers have turned their attention to the problem of unravelling the constitution of rottlerin (see literature) but the formulas for which, in the main, today there appears to be experimental foundation are due to Robertson and to Brockmann. Rottlerin itself is a chromene but is easily converted into compounds of flavone nature.


COCH₃
H₃C
H₃C
C
O
HO
OH
H₃C
CH₂
OH
COCH=CH–C₆H₅
OH

(Robertson[200])

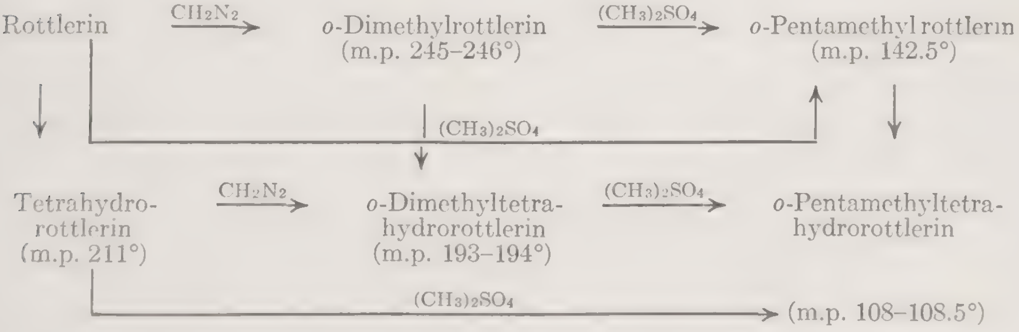

COCH₃
COCH=CH–C₆H₅
O
CH₃
C
CH₃
HO
OH
HO
H₃CO
CH₂
OH
OH

(Brockmann)


O
C
H₃C
CH₂OH
OH
C
C–CH₃
O
CH₃
C
CH₃
HO
C₆H₅CH=CH–CO
OH

The collected results of the work of Brockmann and Maier are probably the easiest to follow, although many of the essential points were known from previous work, but were wrongly interpreted.

Rottlerin gives a pentaacetyl compound, $C_{30}H_{23}O_8(COCH_3)_5$ (m.p. 211.5-212.5°). The results of methylation and hydrogenation experiments are summarized in the following scheme:


Rottlerin
CH₂N₂
o-Dimethylrottlerin
(m.p. 245–246°)
(CH₃)₂SO₄
o-Pentamethyl rottlerin
(m.p. 142.5°)
(CH₃)₂SO₄
Tetrahydro-rottlerin
(m.p. 211°)
CH₂N₂
o-Dimethyltetra-hydrorottlerin
(m.p. 193–194°)
(CH₃)₂SO₄
o-Pentamethyltetra-hydrorottlerin
(CH₃)₂SO₄
(m.p. 108–108.5°)

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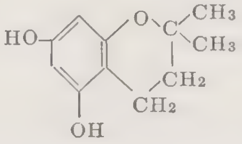
[200] Robinson interprets Robertson's experiments by the formula: [Narang, Ray, Roy: *Chem. Zentr.*, 1939, I, 687.]

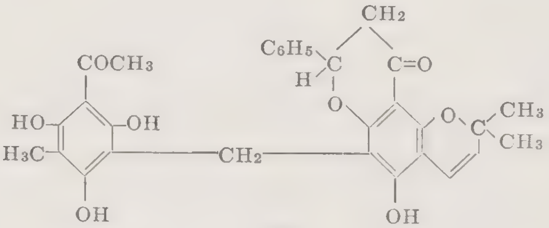
With hydrogen peroxide cinnamic acid is obtained and ozonolysis yields 0.7-0.9 mol. of benzaldehyde which originates as a cinnamic residue bound to a phloroglucinol nucleus as a chalkone. The results of Dutt, who claimed to have isolated phthalic and terephthalic acids by oxidative degradation, were not corroborated and the formula based on these observations can thus no longer be considered. Degradation by the method of Boehm[201] with diazoaminobenzene afforded 3-acetyl-5-methyl-2,4,6-trihydroxyazobenzene, and thermal decomposition yielded methylphloracetophenone.

After Robertson had obtained hydrocinnamic acid and 5,7-dihydroxy-2,2-dimethylchromane by alkali fission of tetrahydrorottlerin, the suggestion of the two formulas above became possible.



5,7-Dihydroxy-2,2-dimethylchromane

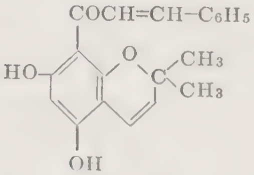
By treatment with alcohol rottlerin is converted into isorottlerin which is formulated as (IX)



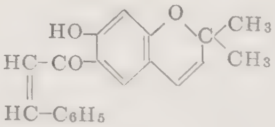
(IX)

and corresponds in origin to the conversion of an *o*-hydroxychalkone into a flavanone.[202]

Rottlerone, $C_{20}H_{18}O_4$ [m.p. 236° (Robertson)], is a compound obtained by treating rottlerin with baryta or sodium carbonate:



or



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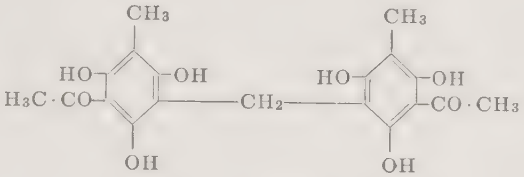
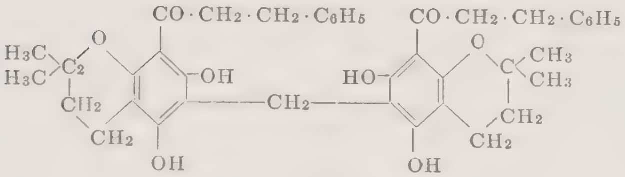
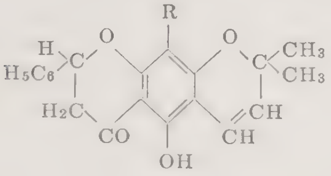
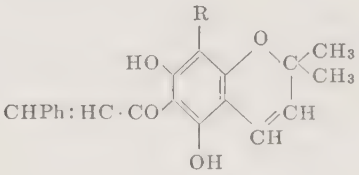
[201] Boehm: *Ann.*, 318, 253 (1901).
[202] Brockmann, Maier: *Naturwiss.*, 27, 259 (1939).

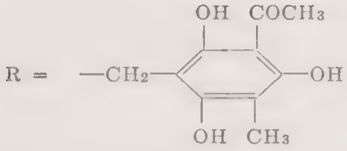
The detail of this work recently assumed a modified significance when it was realized that "tetrahydrorottlerone," which for convenience had been used in place of rottlerone itself, is actually octahydrorottlerone. The formation of octahydrorottlerone from tetrahydrorottlerin is accompanied by the formation of the diarylmethane (A). This fact suggested that octahydrorottlerone is a diaryl compound (B), a suggestion soon confirmed by its synthesis from the appropriate chromane and formaldehyde.


(A)


(B)


(C)


(D)



Robertson and his co-workers [202a] now believe that the formation of isorottlerin is much more complicated than the simple cyclization favored by Brockmann. They prefer structure (C) for isorottlerin. It is regarded as owing its formation to opening of the chromene ring followed by cyclization in the alternative manner as at (D), and then by a chalkone-flavone cyclization. (D) is held to represent the compound allorottlerin, derivatives of which are obtained by hydrogenation of isorottlerin.

[202a] McGookin, Robertson, Tittensor, *J. Chem. Soc.*, 1939, 1579, 1587.

Rottlerin is isolated by first extracting the drug with ether, which removes a large proportion of resin but only a little pigment, and then taking up the major part in benzene (Telle, Dutt), although carbon disulfide may also be used (A. G. Perkin); 100-120 grams of rottlerin are obtained from 1 kg of kamala.[203]

Coloring matters of safflower (bastard saffron) (Carthamin and safflower yellow). Safflower consists of the dried flowers of the bastard saffron, *Carthamus tinctorius,* one of the family of *Cynarocephaleae* native to southern Asia but cultivated in almost all parts of the world. The flowers are either dried or triturated with water immediately after gathering to remove the tinctorially valueless safflower yellow. The red pigment of the plant, carthamin, is difficultly soluble in water but may be extracted with sodium carbonate, precipitated with dilute acid and used directly. Purer products are obtained by removing the pigment on cotton from slightly acid solution, treating the dyed cotton with soda solution and again precipitating the coloring matter. Safflower carmine so obtained comes into commerce as a thick red paste which gives cherry-red direct dyeings on silk and cotton. Among other carthamin preparations may be mentioned rouge en tasses, assiettes, feuilles and compositions of talc with fard de la chine. The dyeings, however, are fugitive to light and air and sensitive toward alkali, chlorine and sulfurous acid, so that carthamin has been almost completely supplanted by synthetic dyestuffs.

Very little is known of the constitution of safflower yellow.[204] An aqueous solution may be precipitated with lead acetate, the lead salt decomposed by sulfuric acid and the solution evaporated in the absence of air; an amorphous residue possessing acid properties is then obtained. It oxidizes and becomes brown in the air and simultaneously loses to some extent its solubility in water. Schlieper gives the empirical molecular formula as $C_{16}H_{20}O_{11}$ and A. G. Perkin [205] was of the opinion that the compound possesses glycosidic character, because on boiling with dilute sulfuric acid a crystalline yellow ether-soluble pigment was obtained, although only in small quantity.

Schlieper originally assigned the empirical formula $C_{14}H_{16}O_7$ to carthamin, describing it as a brick-red powder.[206] On subsequent purification by extracting with pyridine scarlet-red prismatic needles (m.p. 228-230°) were obtained in a yield of 0.3-0.6 per cent. Molecular weight determinations then supported the formula $C_{25}H_{24}O_{12}$. Carthamin con-

[203] Dyeing technique: Hummel, Perkin: *J. Soc. Chem. Ind.*, **14**, 460 (1895); Perkin: *Ibid.*, **19**, 519 (1900).

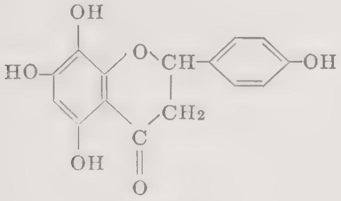
[204] Schlieper: *Ann.*, **58**, 357 (1846); Malin: *Ann.*, **136**, 117 (1865).

[205] A. G. Perkin and A. E. Everest: "The Natural Colouring Matters," p. 594.

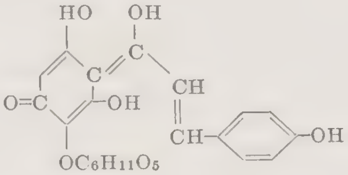
[206] Kametaka, A. G. Perkin: *J. Chem. Soc.*, **97**, 1415 (1910).

tains no methoxyl groups, yields picric acid on treating with nitric acid, *p*-hydroxybenzoic acid with fused caustic potash, and *p*-cumaric acid and *p*-hydroxybenzaldehyde on boiling with alkali. A more recent investigation by Kuroda[207] has shown that a second compound, isocarthamin (m.p. 228°), is extracted from Chinese starting material by dilute hydrochloric acid. Both carthamin and isocarthamin exist in the form of glucosides and yield 1 mol. of glucose on heating with dilute mineral acid. Carthamin is now given the formula $C_{21}H_{22}O_{11} . H_2O$; and isocarthamin, which gradually reverts to carthamin, is formulated in the freshly prepared condition as $C_{21}H_{22}O_{11} . 2H_2O$. The action of hot dilute phosphoric acid on carthamin affords carthamidin, $C_{15}H_{12}O_6$ (very unstable orange-yellow needles, m.p. 218°), and isocarthamidin, $C_{15}H_{12}O_6$ (yellow prisms, m.p. 238°).

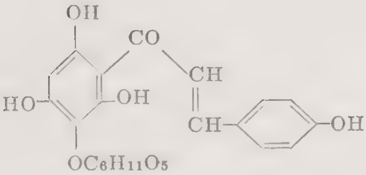
Catalytic reduction of carthamin gave a hydrocarthamin which underwent alkali fission into *p*-hydroxycinnamic acid and a phenol. Carthamin and isocarthamin are provisionally assigned the following constitutions of the chalkone type:



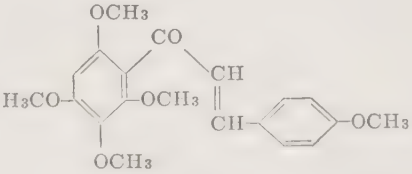
Carthamidin



Carthamin



Isocarthamin



(X)

These constitutions are in agreement with color reactions and the products of methylation; particularly strong support is provided by the conversion of β-acetylcarthamidin (a pentaacetyl derivative of isocarthamin but containing no glucose residue) by means of dimethyl sulfate into 2,3,4,6,4′-pentamethoxychalkone (X) which was synthesized from *p*-methoxybenzaldehyde and 2,3,4,6-tetramethoxyacetophenone.[208]

Centaureidin,[209] $C_{18}H_{16}O_8$ (yellow needles, m.p. 230°), occurs as its glucoside centaurein, $C_{24}H_{26}O_{13}$ (pale-yellow powder, m.p. about 168-

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[207] Kuroda: *Proc. Imp. Acad. Tokyo*, **5**, 32, 82, 86 (1929).

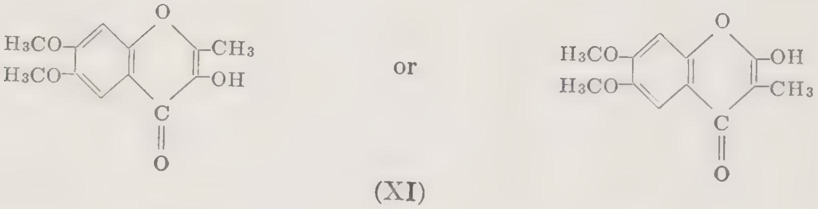
[208] cf. Bargellini, Zoras: *Gazz. chim. ital.*, **64**, 202 (1934).

[209] Bridel, Charaux: *Compt. rend.*, **175**, 833, 1168 (1922); *J. Pharm. Chim.* (7), **27**, 409 (1923).

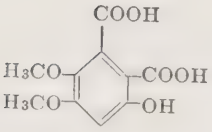
175°), in the root-bark of *Centaurea Jacea,* the common knap-weed. The glucoside may be hydrolyzed to give glucose and a pigment containing three methoxyl groups and regarded as a flavone derivative. It is isolated from an alcoholic extract of the root, 100 grams of root-bark providing 2.6 grams of glucoside.

Citromycetin,[210] $C_{14}H_{20}O_7$ (pale-yellow needles decomposing at 283-285°), is a compound isolated from cultures of various species of *Citromyces.* It behaves as a dibasic acid and also furnishes a diacetyl compound (colorless needles, m.p. 223-224°). On boiling with dilute acid, carbon dioxide is lost with the formation of citromycin, $C_{13}H_{10}O_5$ (yellow needles, m.p. 285-290°), which behaves as a monobasic acid.

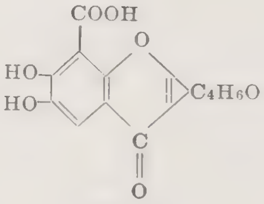
Citromycetin is degraded by caustic potash to what is probably 3,5,6-trihydroxyphthalic acid, and *o*-dimethylcitromycetin yields dimethoxymethylbenzpyrone (XI) and dimethoxyhydroxyphthalic acid (XII) with alcoholic potash, on the basis of which observations constitution (XIII) is proposed for citromycetin:



(XI)



(XII)



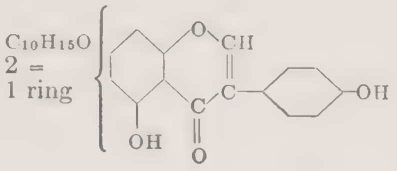
(XIII)

Osage-Orange Pigments. Extraction of the dried meal of the fruit of the osage orange (*Maclura pomifera* Raf.) with ether gave two pigments, *osajiin* and *pomiferin,* present in approximately equal amounts. The two are separated by crystallization from xylene in which the former is the more soluble.

Osajiin, m.p. 189°, has the composition and molecular weight corresponding to $C_{24}H_{24}O_5$; it gives yellow solutions and a green coloration with ferric chloride. Osajiin contains two phenolic groups, and two further oxygen atoms were at first placed in a lactone ring. On controlled catalytic reduction di-, tetra-, and hexa-hydro derivatives were obtained, the first two giving color reactions similar to osajiin itself. These results

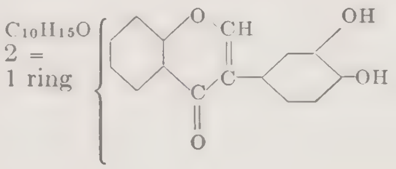
[210] Raistrick: *Philos. Trans. Roy. Soc. London (B),* 220, 1 (1931).

indicated rather a flavone or isoflavone nucleus together with two additional and unconjugated double bonds. Comparison with genistein and pseudobaptigenin favored an isoflavone formulation; and this was confirmed by the isolation of formic acid among the products of alkali degradation. A second significant cleavage product (from osajiin dimethyl ether) was *p*-methoxyphenylacetic acid, for arylacetic acids are to be expected from the fission of isoflavones. From the general similarity to genistein and pseudobaptigenin the following partial formula is suggested for osajiin:



Osajiin and many of its derivatives may be isomerized (*e.g.*, with sulfuric acid), though structural interpretation of these facts is lacking.

Pomiferin, $C_{25}H_{24}O_6$, m.p. 200.5°, is a yellow pigment closely resembling osajiin but containing one more phenolic hydroxylic group. As with osajiin, di-, tetra-, and hexa-hydro derivatives have been obtained and many isomerized. Again an isoflavone structure is indicated; and as the characteristic fission product (from the trimethyl ether) is 3:4-dimethoxyphenylacetic acid the following partial structure is proposed[210a]:



Pigments of Didymocarpus pedicellata.

The following compounds, probably chalkone derivatives, have been isolated from *Didymocarpus pedicellata,* one of the *Gesneriaceae* found in the western subtropical Himalayas and used as a medicinal.[211]

Pedicin, $C_{18}H_{18}O_6$ (orange-red tablets, m.p. 145°). This pigment, which is obtained in 1 per cent yield, forms a phenylhydrazone and a dibenzoyl derivative and takes up two hydrogen atoms on catalytic reduction.

[210a] Walter, Wolfrom, Hess, *J. Am. Chem. Soc.*, **60,** 574 (1938); Wolfrom, Benton, Gregory, Hess, Mahan: *Ibid.*, **61,** 2832 (1939); Wolfrom, Gregory: *Ibid.*, **62,** 651 (1940); Wolfrom, Morgan, Benton: *Ibid.*, **62,** 1484 (1940); Wolfrom, Benton, Gregory, Hess, Mahan, Morgan: *Ibid.*, **63,** 422 (1941); Wolfrom, Mahan, Morgan, Johnson: *Ibid.*, **63,** 1248 (1941); Wolfrom, Mahan: *Ibid.*, **63,** 1253 (1941).

[211] Siddiqui: *J. Indian Chem. Soc.*, **14,** 703 (1937).

Isopedicin, $C_{18}H_{18}O_6$, yellowish prisms, m.p. 106°; yield 0.4 per cent.

Pedicinin, $C_{16}H_{12}O_6$ (carmine red prisms, m.p. 203°). Bose and Dutt [211a] consider the substance to be a dibasic acid.

Pedicellin, $C_{20}H_{22}O_6$ (colorless plates, m.p. 98°), contains five methoxyl groups; yield 1 per cent.

In another communication [211b] tentative structures for these pigments were suggested and two further pigments were reported.

Pseudoisopedicin, $C_{18}H_{18}O_6$, m.p. 126°; this is a saturated compound and passes into pedicin on standing with alkali.

Pedicidin, $C_{37}H_{36}O_{11}$, m.p. 190°; pedicidin is unsaturated and resembles isopedicin in its color reactions.

Cacao red.[212] This pigment occurs to the extent of 1.8-2 per cent in cocoa beans which have not been defatted. It forms an amorphous red powder and resembles tanning red and phlobaphescene. It undergoes slow fission by alkali; acetic acid, protocatechuic acid and what is presumably 2,4,6-trihydroxy-1,3-dimethylbenzene have been detected among the products. Cacao red would thus appear to be a derivative of 3′,4′,3,5,7-pentahydroxy-6,8-dimethyl-2,3-dihydroflavone with the formula $(C_{34}H_{31}O_{10})_x$.

Salinigrico flavonoloside [213] is a pigment (m.p. 292°) occurring in *Salinigricans.* It may be degraded to glucose, rhamnose and salinigrico flavanol (yellow needles, m.p. 208-209°), fusion of which with alkali yields protocatechuic acid.

Pyrilium Pigments (Anthocyans)

Anthocyanins or more generally anthocyans [214] are coloring matters which are responsible for the red, violet and blue pigmentations of flowers, of many fruits and to some extent of other plant tissues. They are all derivatives of 2-phenylbenzopyrilium salts (I) [215] and all, with the exception of a few amino- derivatives, are hydroxy- derivatives existing in the plant usually as glycosides.[216]

[211a] Bose, Dutt: *J. Indian Chem. Soc.*, **17**, 499 (1940).

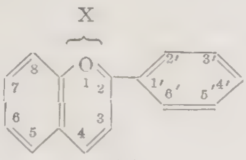
[211b] Sharma, Siddiqui, *Ibid.*, **16**, 423 (1939).

[212] Heiduschka, Bienert: *J. prakt. Chem. (2)*, **117**, 262 (1927); **119**, 199 (1928).

[213] Rabaté: *Bull. soc. chim. biol.*, **17**, 319 (1935).

[214] This name was derived by Marquart (Marquart and Clamor, "Die Farbe der Blüten," Bonn, 1835) from *anthos* and *cyanosis.* According to current usage the whole group of pyrilium pigments is termed anthocyans. Anthocyanins refers specially to glycosides. Earlier monographs: Onslow: "The Anthocyanine Pigments of Plants," 2nd ed., Cambridge University Press, 1925; also Wheldale: "The Anthocyanine Pigments of Plants," Cambridge University Press.

[215] Willstätter, Everest: *Ann.*, **401**, 189 (1913); Willstätter, Nolan: *Ann.*, **408**, 1 (1915); Willstätter, Bolton: **412**, 113 (1916); cf. compilative review (lecture) Willstätter, Schmidt: *Ber.*, **57**, 1946 (1924): "Die Untersuchung der Blüten-Beeren- und Wurzel-farbstoffe ist ein Torso; die grösser angelegte Arbeit ist nämlich durch den Kriegsausbruch unterbrochen und dann mit weitgehender Einschränkung unvollkommen zu Ende geführt worden." R. Robinson and Gertrude M. Robinson have been among the foremost in later research with their determination of the mode of combination of the sugar groups, syntheses of the most important anthocyanins and anthocyanidins and by their systematic examination of plants for their anthocyan content. See R. Robinson: *Ber.*, **67**, A, 85 (1934) (lecture) and R. Robinson, G. M. Robinson: *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932); R. Robinson: *Nature,* **135**, 732 (1935); *Ibid.*, **137**, 172 (1936).

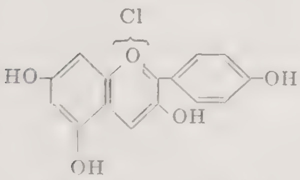


(I)

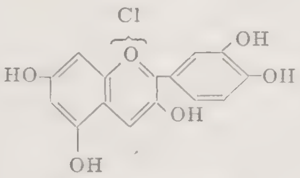
Anthocyans may be hydrolyzed to yield a sugar compound (pentose, hexose, biose) and an aglucone, an anthocyanidin; frequently there is also present a third constituent of the complex, an organic acid such as *p*-hydroxybenzoic, malonic, *p*-hydroxycinnamic or *p*-coumaric acid, which may esterify the hydroxyl groups of the anthocyanidin or those of the sugar constituent.

For long, all the anthocyans found in nature fell into three fundamental types, pelargonidin, cyanidin and delphinidin. These all belong to the flavonol group and all carry a hydroxyl group on carbon atom 3; only recently has an anthocyan, gesnerin with the aglucone gesneridin[217] been found which lacks this feature. Again, a nitrogenous anthocyan, betanin,[218] occurs in the red beet, and a similar compound appears in *Bougainvillaea*.[219] Abnormal types have also been found in *Celosia cristata* and *Atriplex hortensis,* in *Papaver alpinum, Papaver nudicaule*[220] (yellow anthocyan), Iceland poppy, beech-acorn, yew, black-thorn, and *Peltogynum* varieties.[221]

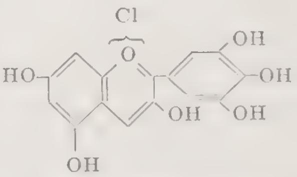
At present, therefore, four fundamental types are known; trihydroxy- (gesneridin), tetrahydroxy- (pelargonidin), pentahydroxy- (cyanidin) and hexahydroxy- (delphinidin) flavylium derivatives; a methoxy derivative, peonidin, derived from cyanidin, and three methoxy derivatives of delphinidin, petunidin, malvidin (syringidin) and hirsutidin have been found in nature. The most important basic types are set out below:



Pelargonidin chloride



Cyanidin chloride



Delphinidin chloride

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[216] Occurrence of free anthocyanidines: Hadders, Wehmer in Klein, "Handbuch der Pflanzenanalyse," III, 2. p. 988; Price, Sturgess: *Biochem. J.*, 32, 1658 (1938).

[217] G. M. Robinson, R. Robinson: *Nature,* **130,** 21 (1932); *Biochem. J.*, **26,** 1647 (1932).

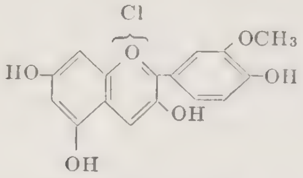
[218] Schudel: *Dissertation,* Zurich, 1918 (carried out with Willstätter).

[219] G. M. Robinson, R. Robinson: *Biochem. J.*, **26,** 1647 (1932).

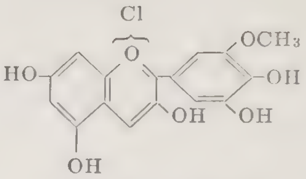
[220] R. Robinson: *Ber.*, **67,** A, 103 (1934).

[221] G. M. Robinson, R. Robinson: *Nature,* **130,** 21 (1932); *Biochem. J.*, **27,** 296 (1933).

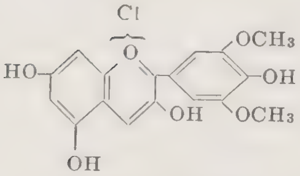
From these are derived:



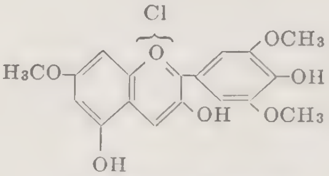
Peonidin chloride



Petunidin chloride

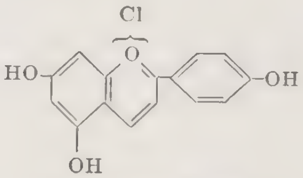


Malvidin Chloride



Hirsutidin Chloride

There remains the special type:



Gesneridin chloride

The pigments are written here as chlorides and they are usually isolated in this form, although existing in the plant in association with the plant acids. The isolation of anthocyans is normally based on the low solubility of their oxonium salts with picric acid,[222] hydrochloric acid, etc.; specific procedures are given for individual pigments.[223] One plant will frequently contain mixtures of anthocyans, the separation of which presents no little difficulty. Hydrolysis of the anthocyans to sugar-free pigments [224] is effected by boiling for a short time with hydrochloric acid or in particular instances with alkalies. More recently, detailed procedures [225] have been described by which the anthocyan or anthocyanidin content of plant tissues may be rapidly and exactly examined. Finally the elegant method of chromatographic analysis [226] has been applied to the purification of anthocyans.

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[222] Willstätter, Schudel: *Ber.*, **51**, 782 (1918).

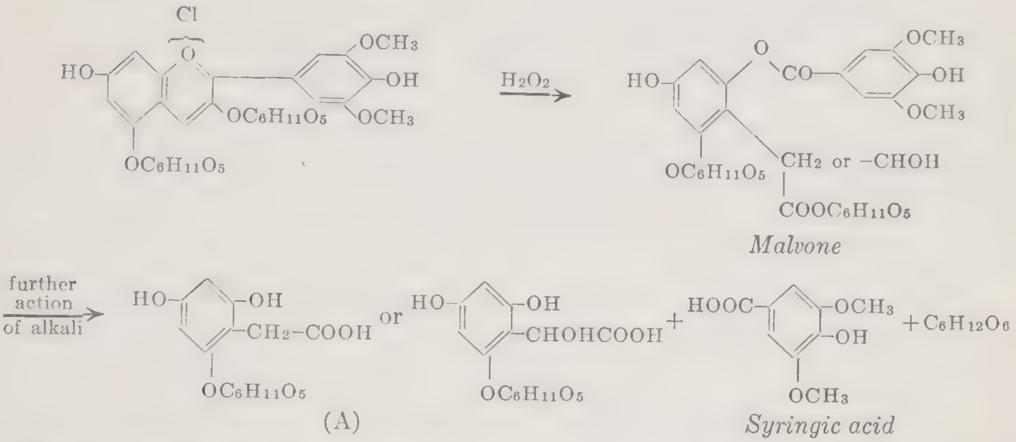
[223] Cf., for example, Willstätter, Mieg: *Ann.*, **408**, 123 (1915); Willstätter, Nolan: *Ann.*, **408**, 137 (1915); Willstätter, Zollinger: *Ann.*, **408**, 83 (1915); **412**, 195 (1917); Karrer, Widmer: *Helv. Chim. Acta*, **10**, 9, 69 (1927).

[224] Cf., for example, Willstätter, Everest: *Ann.*, **401**, 226 (1913); Willstätter, Mieg: *Ann.*, **408**, 75 (1915); Karrer, Widmer: *Helv. Chim. Acta*, **10**, 72 (1927).

[225] G. Robinson, R. Robinson: *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932); **27**, 206 (1933); **28**, 1712 (1934). It would seem useless to quote individual preparations of anthocyans or anthocyanidins, as close adherence to each procedure is necessary. As far as possible the literature has been carefully collected.

[226] Karrer, Strong: *Helv. Chim. Acta*, **19**, 25 (1936).

The constitutions indicated in the formulas given above have been determined by degradation. Anthocyans[227] and anthocyanidins which contain no adjacent hydroxyl groups in the phenyl part of the molecule are degraded by 15-per cent hydrogen peroxide, as is illustrated by the case of malvin:



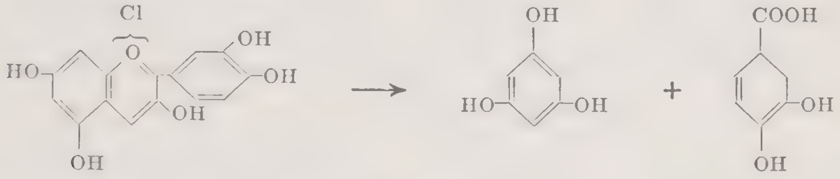
Malvone

(A)

Syringic acid

The hydrolytic fragment (A) containing the phloroglucinol nucleus has not yet been isolated, so that it is still impossible to decide whether malvone contains the —CH_2— or —CH(OH)— group.

On fusion with alkali[228] phenolic and acid fission products are obtained, *e.g.*, phloroglucinol and a hydroxyacid, whilst methoxyl groups are not infrequently hydrolyzed:



Degradation by baryta water[229] or dilute caustic soda possesses the advantage that, while the fission is similar, the methoxyl groups are not hydrolyzed and their orientation in the anthocyan molecule may consequently be deduced from the structure of the methoxy acids formed.

Turning now to syntheses,[230] the oldest, the interaction of methoxycumarins with aryl magnesium compounds,[231] is of only limited application:

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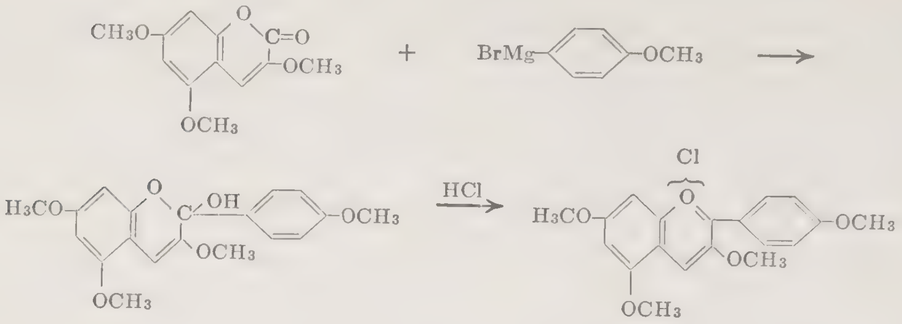
[227] Karrer, Widmer, Helfenstein, Hürlimann, Nievergelt, Monsarrat-Thoms: *Helv. Chim. Acta,* **10,** 729 (1927); Karrer, de Meuron: *Ibid.,* **15,** 507 (1932).

[228] Willstätter, Mallison: *Ann.,* **408,** 40 (1915); Willstätter, Bolton: *Ann.,* **408,** 59 (1915).

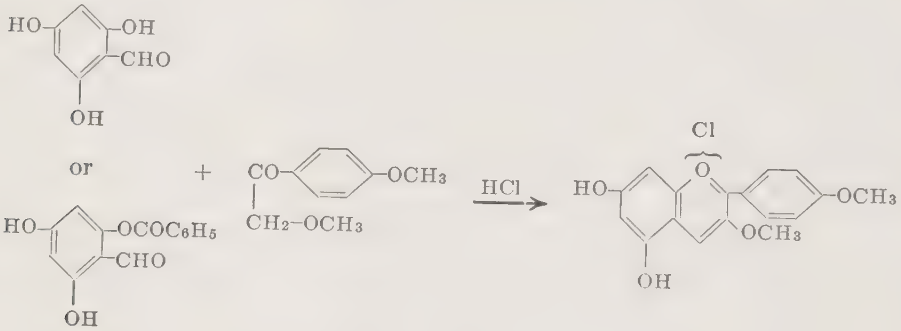
[229] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 20. 29 (1927).

[230] cf. Robinson: "Über die Synthese von Anthocyaninen" (lecture), *Ber.,* **67A,** 85 (1934).

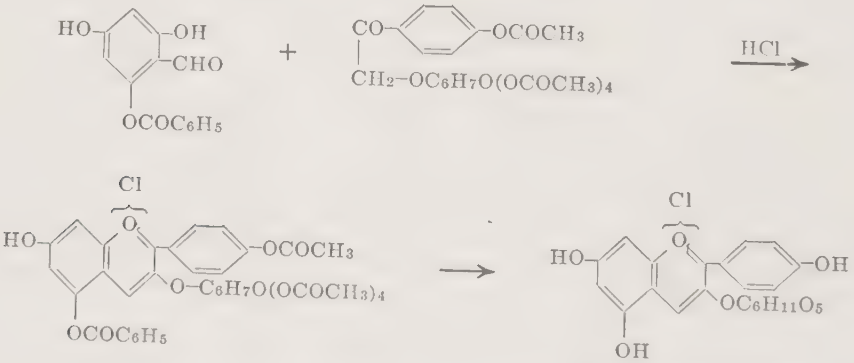
[231] Decker, v. Fellenberg: *Ber.,* **40,** 3815 (1907); *Ann.,* **356,** 281 (1907); Willstätter, Zechmeister: *Sitzber. Kgl. preuss. Akad. Wiss. (Berlin),* **1914,** 886; cf. also Willstätter: *Ber.,* **47,** 2865 (1914); Willstätter, Zechmeister, Kindler: *Ber.,* **57,** 1938 (1924); Willstätter, Schmidt: *Ber.,* **57,** 1945 (1924).



Certain anthocyanidins may be synthesized by the condensation[232] of *o*-hydroxyaldehydes with acetophenone derivatives, *e.g.*,



It is to be noted that the readiness with which a phloroglucinol aldehyde condenses with itself makes it necessary to employ the usual protective methods, such as methylation, acetylation or benzoylation. By this same general method anthocyanins may be obtained by starting with, *e.g.*, tetraacetyl-β-glucosidoacetophenone derivatives[233]:

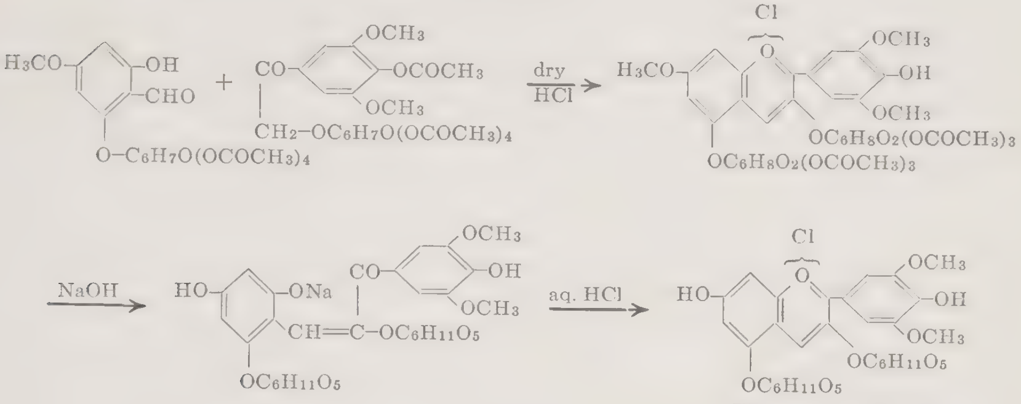


Callistephin chloride

[232] Perkin, Robinson, Turner: *J. Chem. Soc.*, **93**, 1085 (1908); Pratt, Robinson: *Ibid.*, **121**, 1577 (1922); **123**, 745 (1923); **125**, 188 (1924); Pratt, Robinson, Williams: *Ibid.*, **125**, 199 (1924); Robinson, Lawson, Crabtree, Das, Lunt, Roberts, Williams: *Ibid.*, **125**, 207 (1924); Ridgway, Robinson: *Ibid.*, **125**, 214 (1924); Pratt, Robinson: *Ibid.*, **127**, 166, 1128, 1182 (1925); Robinson, León: *Anales soc. españ. fís. quím.*, **29**, 415 (1931); *J. Chem. Soc.*, **1931**, 2732.

[233] Robertson, Robinson: *J. Chem. Soc.*, **1928**, 1460; Fonseka, Robinson: *Ibid.*, **1931**, 2730.

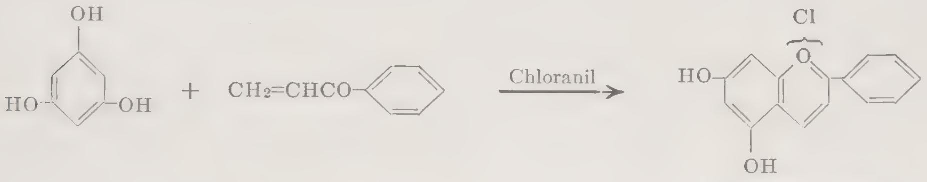
Diglycosides may be obtained in a similar manner, as is instanced by the following synthesis of the 3,5-diglucoside hirsutin[234]:


H3CO
OH
CHO
O–C6H7O(OCOCH3)4
+
CO
OCH3
OCOCH3
OCH3
CH2–OC6H7O(OCOCH3)4
dry HCl
Cl
O
H3CO
OCH3
OH
OCH3
OC6H8O2(OCOCH3)3
OC6H8O2(OCOCH3)3
NaOH
HO
ONa
CO
OCH3
OH
OCH3
CH=C–OC6H11O5
OC6H11O5
aq. HCl
Cl
O
HO
OCH3
OH
OCH3
OC6H11O5
OC6H11O5

Hirsutin chloride

In recent work there is a tendency[235] to dispense with the protective acetyl groups.

Another synthesis[236] which has hitherto been applied only to the synthesis of chrysinidin (a pyrilium salt corresponding to the anthoxanthidin chrysin, which has not yet been found in nature) consists in the interaction of phenols with reactive ketones:


OH
HO
OH
+
CH2=CHCO
Chloranil
Cl
O
HO
OH

Chrysinidin

It may be mentioned here that the examination of absorption spectra[237] is of considerable value in work on anthocyanins, and that a number of regularities have been observed.

Monoglycosides have also been obtained by Willstätter[238] by the partial hydrolysis of diglycosides. Thus pelargonin, the 3,5-diglucoside of pelargonidin, yielded a monoglucoside later recognized as the 5-derivative, to which the name *pelargonenin* was assigned.

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[234] Robinson, Todd: *Ibid.*, **1932**, 2293; syntheses of reso-anthocyanins (resorcinol in place of phloroglucinol): Grove, Levy, Nair, Robinson: *Ibid.*, **1934**, 1614; 6-hydroxy-derivatives of the chief anthocyanidins: Charlesworth, Robinson: *Ibid.*, **1934**, 1619; other syntheses: Healey, Robinson: *Ibid.*, **1934**, 1625.

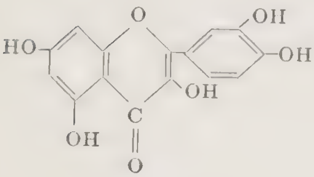
[235] Robinson: *Ber.*, **67A**, 104 (1934).

[236] Robinson, Walker: *J. Chem. Soc.*, **1934**, 1435; **1935**, 941.

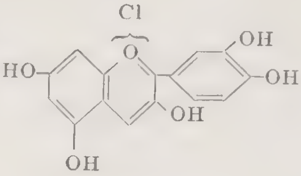
[237] Karrer in Klein, "Handbuch der Pflanzenanalyze," **III**, 2, p. 956, and Hayashi: *Acta phytochimica*, **7**, 117, 143 (1933); **8**, 65 (1934).

[238] Willstätter, Bolton: *Ann.*, **412**, 133 (1917); syntheses of monoglycosides: León, Robertson, Robinson, Seshadri: *J. Chem. Soc.*, **1931**, 2672; León, Robinson: *Ibid.*, **1932**, 2221.

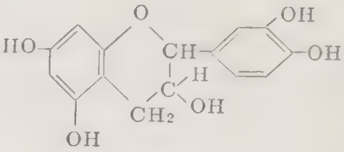
The relationship between anthocyanidins, flavonols and epicatechins may be appreciated from the following constitutional formulas:



Quercetin



Cyanidin



Epicatechin

It has proved possible to convert quercetin into cyanidin [239] as well as many other flavone derivatives into anthocyanidins, to convert cyanadin into epicatechin [240] (now termed cyanidol), fisenitidin into fisenitidol (hypothetical quebrachocatechin) and butinidin into butinidol, and also to effect the reverse conversion of flavylium salts into flavones.[241] It is certain that the three classes of compounds are closely interrelated in the plant, but the anthocyanidins corresponding to the anthoxanthidins are almost exclusively synthetic compounds and have not so far been found to any extent in the plant.

In the following account the corresponding anthoxanthidin is given in brackets: chrysinidin [242] (chrysin), galanginidin [243] (galangin), apigenidin [244,245] (apigenin), acacetinidin [244,245] (acacetin), butinidin [246] (butin), luteolinidin [244,247] (luteolin), lotoflavinidin [248] (lotoflavin), fisetinidin [249] (fisetin), morindin [250] (morin), rhamnetinidin [251] (rhamnetin) and datiscetinidin [252] (datiscetin).

Anthocyans often occur in the plant in a red, violet or blue form; the red form is regarded as the oxonium salt. On conversion into their alkali compounds inner oxonium salts derived from an oxonium base may be assumed to arise (in these formulas the glucose residues are omitted):

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[239] Willstätter, Mallison: *Sitzber. Kgl. preuss. Akad. Wiss. (Berlin)*, **1914**, 769; Asahina, Inubuse, Nakagome: *Ber.*, **62**, 3016 (1929); Asahina, Inubuse: *Ibid.*, **64**, 1256 (1931); Kondo: *J. Pharm. Soc. (Japan)*, **52**, 47 (1932).

[240] Freudenberg, Fikentscher, Harder, Schmidt: *Ann.*, **444**, 135 (1925); Freudenberg, Maitland: *Ann.*, **510**, 193 (1934); Freudenberg. Karimullah, Steinbrunn: *Ann.*, **518**, 37 (1935); cf. Chater: *Chem. Zentr.*, **1935**, II, 1299; Reichel, Burkart: *Ann.*, **536**, 164 (1938).

[241] Robinson, Schwarzenbach: *J. Chem. Soc*. **1930**, 822.

[242] cf. Bülow, v. Sicherer: *Ber.*, **34**, 3889 (1901); Pratt, Robinson, Williams: *J. Chem. Soc.*, **125**, 199 (1924); Pratt, Robinson: *Ibid.*, **127**, 1128 (1925); Pratt, Robinson, Robertson: *Ibid.*, **1927**, 1975.

[243] Malkin, Robinson: *Ibid.*, **127**, 1190 (1925); Pratt, Robinson: *Ibid.*, **127**, 1128 (1925); Willstätter, Schmidt: *Ber.*, **57**, 1945 (1924).

[244] Pratt, Robinson, Williams: *J. Chem. Soc.*, **125**, 199 (1924); Pratt, Robinson: *Ibid.*, **127**, 1128 (1925).

[245] Pratt, Robinson, Robertson: *Ibid.*, **1927**, 1975.

[246] Robertson, Robinson: *Ibid.*, **1926**, 1951.

[247] Pratt, Robinson: *Ibid.*, **127**, 1128 (1925); León, Robinson: *Ibid.*, **1931**, 2732.

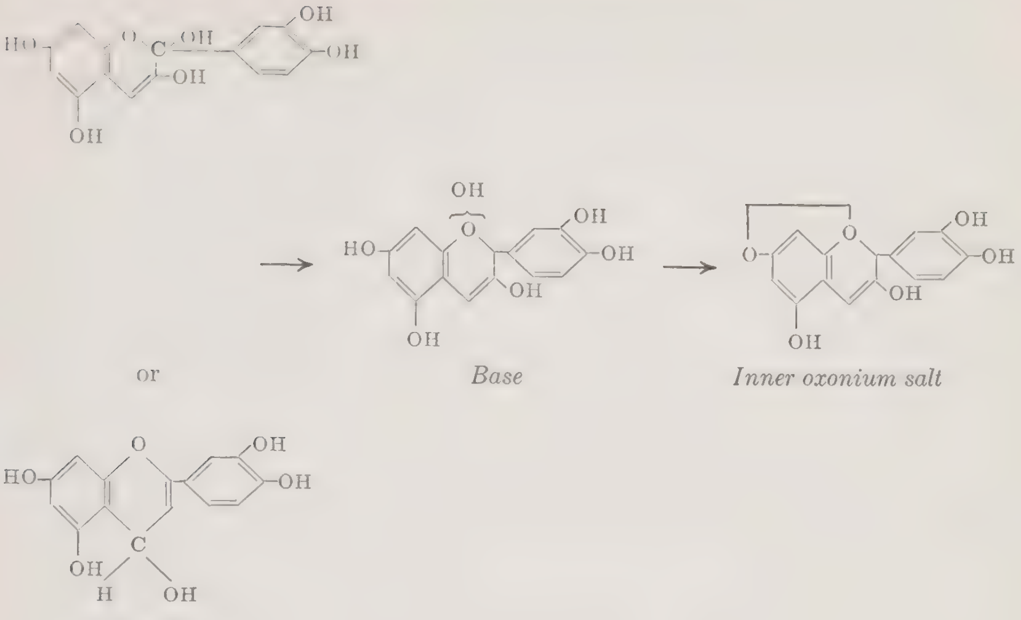
[248] Pratt, Robinson: *Ibid.*, **127**, 1128 (1925).

[249] Pratt, Robinson: *Ibid.*, **127**, 1128 (1925); León, Robinson: Ibid., **1931**, 2732.

[250] Pratt, Robinson: *Ibid.*, **127**, 1128, 1182 (1925); Willstätter, Schmidt: *Ber.*, **57**, 1945 (1924).

[251] Robertson, Robinson: *J. Chem. Soc.*, **1927**, 2196.

[252] Pratt, Robinson: *Ibid.*, **127**, 1182 (1925).



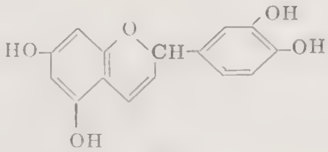
or

Base

Inner oxonium salt

Pseudobase

Moreover, as most anthocyans and anthocyanidins are converted in weakly acid, neutral or particularly in alkaline solution into a colorless modification, it may be assumed that a pseudobase is formed analogous to the carbinol bases of the triphenylmethane dyestuffs. Mineral acids regenerate the oxonium salts. This similarity between triphenylmethane dyestuffs and anthocyans is substantiated by the reduction of cyanidin with zinc dust and pyridine to a leuco- compound [253] which is intermediate between cyanidin and catechin (with respect to the degree of reduction) and to which Kuhn assigns formula (I). Cyanidin is reformed on admitting air. Oxonium salts often undergo a change of color to blue on addition of alkali, and as these blue or violet alkali salts are obtained only from those flavylium salts containing a free hydroxyl group [254] in the 4′-position, constitution (III) must be considered for them rather than the oxonium formulation (II):



(I)

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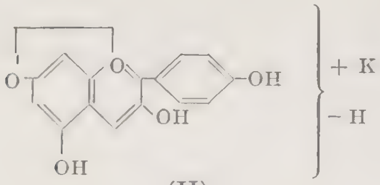
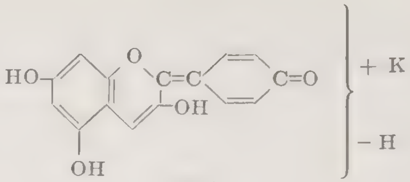
[253] Kuhn, Winterstein: *Ber.*, **65**, 1742 (1932).

[254] Buck, Heilbron: *J. Chem. Soc.*, **121**, 1198 (1922); Dickinson, Heilbron: *Ibid.*, **1927**, 15; Gatewood, Robinson: *Ibid.*, **1926**, 1959.



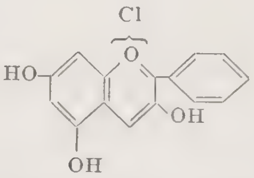
(II)



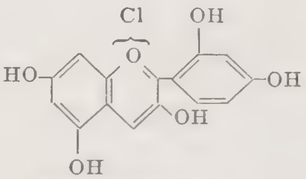
(III)

In actual fact, blue flowers are known to yield more ash than red ones, a fact which agrees with the hypothesis that the blue color is due to alkali salts.[255] At least four hydroxyl groups are necessary in the molecule [256] for the appearance of a pure blue color, this being the case with pelargonidin.

On the other hand the importance of the orientation as well as the number of hydroxyl groups is indicated by comparing the anthocyanidins, galanginidin and morinidin [257]:



Galanginidin chloride



Morinidin chloride

Morinidin resembles in its tinctorial properties the less highly oxygenated pelargonidin rather than its isomeride cyanidin. The introduction of a hydroxyl group in the *meta* position to the 4′-hydroxyl group of pelargonidin has thus a much smaller effect than a similar substitution in the *ortho* position. On the other hand, galanginidin is no longer red in aqueous acid solution, but scarlet-yellow. The color of the anthocyans is thus strongly influenced by the substitution of even one hydroxyl group in the phenyl ring. Galanginidin is sharply distinguished from other anthocyanidins by its inability to form a blue or violet alkali salt, this fact recalling the constitution suggested above for these blue salts. The problem of determining the number and orientation of the sugar residues has given rise to extended researches.[258] The fundamental type is probably a 3-monoside and the replacement of the 5-position is a secondary phenomenon, as evidenced by the fact that no 5-monosides have yet been found in nature; biosides, on the other hand, have been encountered. Among features distinguishing between these groups are color reactions

[255] Karrer, Widmer, Helfenstein, Hürliman, Nievergelt, Monsarrat-Thoms: *Helv. Chim. Acta*, 10, 742 (1927).

[256] Pratt, Robinson: *J. Chem. Soc.*, 125, 188 (1924).

[257] Willstätter, Schmidt: *Ber.*, 57, 1945 (1924).

[258] G. M. Robinson, R. Robinson: *Nature*, 128, 413 (1931); *Biochem. J.*, 25, 1687 (1931); 26, 1647 (1932).

and partition coefficients between amyl alcohol and dilute hydrochloric acid.[259] Moreover these researches have been assisted by the possibility of applying synthetic methods to these glycosides.[260] Thus the preparation of a cyanidin-3,7-diglucoside (MacDowell and Robinson [261]) has revealed that it is only with difficulty distinguished from cyanidin-3,5-diglucoside, so that anthocyans which have hitherto been classified as 3,5-dimonosides may possibly be in reality 3,7-dimonosides. A further elegant method of determining the position of the sugar residues consists in completely methylating the anthocyan, removing the sugar residue by hydrolysis and establishing the positions of the free hydroxyl groups in the aglucone so obtained.[262]

Among sugars which are bound glycosidically in naturally occurring anthocyans may be mentioned glucose, rhamnose and galactose, although others, particularly pentoses, are known to occur. Anthocyans and anthoxanthins appear together in a number of plants, but only in the brown wall-flower, the red rose [263] and the purple-black pansy [264] have the corresponding flavone and flavylium pigments been found in the same tissue. In some flowers, moreover, examination is not facilitated by the presence of anthocyans of types [265] other than those usually found in the same species. Anthocyans [266] occur in the leaves of red varieties of a number of plants (*e.g.*, bloody-beech or hazel), and in young shoots as well as in autumnal foliage, this wide distribution raising the question of their mode of origin.[267]

The wide distribution of flavone derivatives in the plant kingdom originally led to the view that the flavones are precursors [268] of the anthocyans, but it was later demonstrated that colorless precursors [269] of the anthocyans are present in vine-leaves; these generate the pigments in the presence of hydrochloric acid and in the absence of oxygen, and they were regarded as glycosides of leuco-derivatives of the anthocyans proper. Recent research [270] recognizes three broad groups of leucocyanins:

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[259] Willstätter, Zollinger: *Ann.*, **412**, 209 (1917).

[260] cf. Robinson, León: *Anales soc. españ. físi quím.*, **30**, 31 (1932); Karrer, de Meuron: *Helv. Chim. Acta*, **15**, 507, 1212 (1932); Robinson, Todd: *J. Chem. Soc.*, **1932**, 2293, 2299, 2488.

[261] G. M. Robinson, R. Robinson: *Biochem. J.*, **26**, 1647 (1932).

[262] Karrer, Widmer, Helfenstein, Hürliman, Nievergelt, Monsarrat-Thoms: *Helv. Chim. Acta*, **10**, 729 (1927); Karrer, Widmer: *Ibid.*, **10**, 67 (1927); **12**, 292 (1929); Karrer, de Meuron: *Ibid.*, **15**, 507 (1932).

[263] Scott-Moncrieff: *Biochem. J.*, **24**, 753 (1930).

[264] Everest: *Proc. Roy. Soc. (London)*, **90B**, 251 (1918).

[265] Onslow: *Nature*, **129**, 601 (1932).

[266] Systematic review of the occurrence and distribution of the anthocyanins. Hadders, Wehmer in Klein: "Handbuch der Pflanzenanalyse," **III**, 2, p. 984; Lawrence, Price, G. M. Robinson, R. Robinson: *Biochem. J.*, **32**, 1661 (1938); Price, Sturgess: *Biochem. J.*, **32**, 1658 (1938).

[267] Noack: *Z. Botan.*, **14**, 73 (1922).

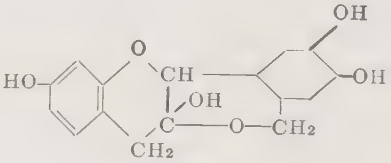
[268] Klein, Werner: *Z. physiol. Chem.*, **143**, 9 (1925).

[269] Rosenheim: *Biochem. J.*, **14**, 178 (1920).

[270] G. M. Robinson, R. Robinson: *Ibid.*, **27**, 206 (1933); **28**, 1712 (1934); *J. Chem. Soc.*, 1935, 744; G. M. Robinson: *Ibid.*, **1937**, 1157; Beale, G. M. Robinson, R. Robinson, Scott-Moncrieff: *J. Genetics*, **37**, 375 (1939); R. Robinson, G. M. Robinson: *J. Am. Chem. Soc.*, **61**, 1605 (1939).

a) those insoluble in water and in the usual organic solvents;
b) those soluble in water but not extracted by ethyl acetate;
c) those soluble in water which are extracted by ethyl acetate.

Class (b) would include simple glycosides or diglycosides. The compounds comprising class (c) are sugar-free, and closer examination has resulted in the isolation of a leucocyanidin, of formula $C_{16}H_{14}O_6$, and probably possessing the constitution (I) from *Peltogyne porphyrocardia, Peltogyne pubescens* and *Copaifera publifera.* The most recent results [271] suggest that the plant may contain flavones or leucoanthocyanins exclusively, or both may occur together; but it must be repeated that although present knowledge is not sufficiently adequate to reveal positively the mode of formation of anthocyans in plants, it is certain that oxidation does not play even a predominant role, and that the so-called leucoanthocyanins are neither pseudobases in the above sense, nor are they easily oxidized anthocyan reduction products such as were obtained by Kuhn.[272]



(I)

It is interesting to note that in some flowers a change of color with change of temperature has been observed, *e.g.*, the flowers of *Myosotis,* which are red at a low temperature, become bright blue in warmer surroundings, and *Erodium* and *Syringa* change from blue-violet to colorless.

The influence of the acidity or alkalinity [273] of the cell-sap on the shade of color in the flower was recognized when the problem of the chemical constitution of the anthocyans was being actively prosecuted, but it is now known that crude extracts contain co-pigments such as tannin, gallic acid, etc. which possess the ability to intensify or modify the color. Thus the glucoside of 2-hydroxyxanthone is an active co-pigment for cyanin, and the inner violet parts of the fuchsia flower contain tannin and are thus distinguished from the external red portions. The formation of complexes with organic materials or, for example, with iron has a greater effect on the color of varying varieties than the acidity of the cell-sap. *Primula sinensis,* in which the magenta and blue varieties

[271] Bancroft, Rutzler, Jr., *J. Am. Chem. Soc.*, **60**, 2738, 2945 (1938); Rutzler, Jr., *Ibid.*, **61**, 1160 (1939).

[272] R. Robinson, G. M. Robinson: *Ibid.*, **61**, 1605 (1939).

[273] Willstätter, Zollinger: *Ann.*, **412**, 195 (1917); G. M. Robinson, R. Robinson: *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932).

contain the same anthocyan, would appear to be an exception, but even here the much more intensely colored magenta specimens may owe their color to a smaller amount of a modifying factor. The co-pigments may also possess the function of stabilizing the anthocyans against photochemical decomposition.

The predominant shades[274] of the anthocyanidins are indicated in the following table:

	Unmordanted wool	Tin-mordanted wool	Tannin-mordanted cotton
Pelargonidin	....	purple-red	blue-red
Cyanidin	rose	blue-violet	violet
Delphinidin	violet	blue-violet	blue-violet

It will be seen that these pigments have, as might be expected, the character of mordant dyestuffs. Their affinity for the thread is good and the dyeings are fast to light but fugitive to washing and soaps; the dyeings weaken on standing due, it may be, to isomerization. The glycosides are tinctorially practically equivalent to the anthocyanidins.

1. **Gesneridin chloride,**[275] $C_{15}H_{11}O_4Cl$ (pale orange-yellow prisms), is identical with 5,7,4′-trihydroxyflavylium chloride (apigenidin chloride) and has been synthesized[276] from 2-*o*-benzoylphloroglucinol aldehyde and *p*-methoxyacetophenone, with subsequent demethylation. It occurs as gesnerin[277] (obtained only in solution) in the orange-red flowers of *Gesnera fulgens (Gesnera cardinalis)* together with what is probably 3′5′-dimethoxygesnerin. Gesnerin[278] has also been synthesized from 4-hydroxyacetophenone and 2-*o*-tetraacetyl-β-glucosidylphloroglucinol aldehyde.

2. **Pelargonidin chloride,** $C_{15}H_{11}O_5Cl$ (3,5,7,4′-tetrahydroxyflavylium chloride, rectangular tablets, four-sided prisms or forked duplets), yields *p*-hydroxybenzoic acid and phloroglucinol[279] on fission by alkali. Synthesis has been effected from trimethoxycumarin and bromanisole[280] and also from 2-*o*-benzoyl-phloroglucinol aldehyde and ω,4-dihydroxyacetophenone.[281]

The following anthocyanins are derived from pelargonidin: Callistephin chloride,[282] $C_{21}H_{21}O_{10}Cl$ (the 3-monoglucoside, fine yellow-red needles), occurring with chrysanthemin in *Callistephus chinensis* (summer aster) and in carnations.[283] Its constitution is established by its synthesis[284] from ω,4-dihydroxyacetophenone, *o*-tetraacetyl-α-glucosidylbromide and 2-*o*-benzoylphloroglucinol aldehyde.

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[274] Willstätter, Nolan: *Ann.*, **408**, 1 (1915).

[275] The pigments are written as chlorides although they are associated with other acids in the plant. Reference may be made to the systematic work of G. M. and R. Robinson [*Biochem. J.*, 25, 1687 (1931); 26, 1647 (1932)] on the mode of occurrence of anthocyans.

[276] Pratt, Robertson, Robinson: *J. Chem. Soc.*, **1927**, 1975; Asahina, Inubuse: *Ber.*, **61**, 1646 (1928).

[277] G. M. Robinson, R. Robinson: *Nature*, **130**, 21 (1932); *Biochem. J.*, **26**, 1647 (1932).

[278] G. M. Robinson, R. Robinson, Todd: *J. Chem. Soc.*, **1934**, 809.

[279] Willstätter, Bolton: *Ann.*, **408**, 59 (1915).

[280] Willstätter, Zechmeister, Kindler: *Ber.*, **57**, 1938 (1924).

[281] Robertson, Robinson, Sugiura: *J. Chem. Soc.*, **1928**, 1533; cf. also Malkin, Robinson: *Ibid.*, **127**, 1190 (1925).

[282] Willstätter, Burdick: *Ann.*, **412**, 149 (1916); Robertson, Robinson: *J. Chem. Soc.*, 1927, 1710, 2196.

Fragasin,[285] the coloring matter of the wild strawberry (*Fragara vesca*) is the 3-galactoside. Pelargonin chloride, $C_{27}H_{31}O_{15}Cl$ (scarlet-red needles, the 3,5-diglucoside), occurs in the flowers of the scarlet pelargonium[286] (*Pelargonium zonale*), in *Centaurea cyanus*, the red cornflower,[287] in *Dahlia variabilis* the scarlet-red dahlia,[287] and in the red-flowered *Pharbitis Nil* (Morning Glory).[288] Pelargonin chloride has been synthesized[289] from 2-*o*-(*o*-monoacetyl-β-glucosidoxy)-4-acetoxyacetophenone.

Monardein (=salvianin)[290] occurs in the flowers of *Monarda didyma* (golden balmmint) and in *Salvia splendens* and *Salvia coccinea.* Its hydrolysis yields *p*-hydroxycinnamic acid, malonic acid and monardin (salvinin), which is identical with pelargonin. One carboxyl group of the malonic acid residue appears to be esterified and it is clear that the formulation provisionally assigned by Karrer must, according to more recent research, be modified with respect to the position of the sugar residue.

Punicin chloride,[291] $C_{27}H_{31}O_{15}Cl$ (orange needles), appears, except for the melting point which is 10-15° higher, to be identical with pelargonin chloride. It occurs in the blossom of *Punica granatum,* the pomegranate tree, and on hydrolysis yields glucose and pelargonidin. The common red or corn poppy contains, in addition to cyanin and mekocyanin chlorides, a third pigment, *mekopelargonin,*[292] the aglucone of which, $C_{15}H_{12}O_6Cl$, appears to be a pelargonidin with firmly bound water of crystallization. It is associated with *p*-hydroxybenzoic acid and a hexose.

3. **Cyanidin chloride,**[293] $C_{15}H_{11}O_6Cl$ (3,5,7,3′,4′-pentahydroxyflavylium chloride, violet-red needles), breaks down by fission with alkali into phloroglucinol and protocatechuic acid.[294] Syntheses have been effected from trimethoxycumarin and iodoveratrole,[295] from ω-methoxyacetoveratrone and 2-hydroxy-4,6-dimethoxybenzaldehyde[296] and from 2-*o*-ben-

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[283] Robinson: *Ber.*, **67A**, 97 (1934).

[284] Robertson, Robinson: *J. Chem. Soc.*, **1928**, 1460.

[285] Robinson: *Ber.*, **67A**, 98 (1934); Nair, Robinson: *J. Chem. Soc.*, **1934**, 1611.

[286] Willstätter, Bolton: *Ann.*, **408**, 42 (1915).

[287] Willstätter, Mallinson: *Ann.*, **408**, 147 (1915).

[288] Kataoka: *Proc. Imp. Acad. Tokyo*, **4**, 389 (1934); *Acta phytochimica (Japan)*, **9**, 35 (1936).

[289] Robinson, Todd: *J. Chem. Soc.*, **1932**, 2488.

[290] Karrer, Widmer: *Helv. Chim. Acta*, **10**, 67 (1927); **11**, 837 (1928); **12**, 292 (1929); cf. Willstätter, Bolton: *Ann.*, **412**, 113 (1917).

[291] Karrer, Widmer: *Helv. Chim. Acta*, **10**, 67 (1927).

[292] Schmid, Huber: *Monatsh.*, **57**, 383 (1931); **60**, 285 (1932); Schmid, Körperth: *Monatsh.*, **68**, 290 (1936).

[293] Willstätter, Everest: *Ann.*, **401**, 227 (1913).

[294] Willstätter, Everest: *Ann.*, **401**, 189 (1913); Willstätter, Nolan: *Ann.*, **408**, 1 (1915); Willstätter, Mallison: *Ann.*, **408**, 15 (1915).

[295] Willstätter, Zechmeister, Kindler: *Ber.*, **57**, 1938 (1924).

[296] Pratt, Robinson: *J. Chem. Soc.*, **127**, 166 (1925).

zoyl-phloroglucinol and ω,3,4-trihydroxyacetophenone.[297] It may be noted also that cyanidin may be obtained from quercetin by reduction.[298]

Pigments Derived from Cyanidin

Chrysanthemin (=asterin) chloride,[299] $C_{21}H_{20}O_{11}Cl$ (3-β-glucosidyl-cyanidin chloride, acute-angled rhombic leaflets which are grey-violet by transmitted light or bordeaux-red powder), occurs in *Aster chinensis*, the summer aster, in *Chrysanthemum indicum*, the winter aster, in the red autumnal foliage [300] of *Acer circumbolatum Maxim*, *Acer ornatum* Carr Matsumurae Nakai and *Acer Sieboldianum* Miqu., in the scarlet-red flowers of *Lycoris radiata*,[301] the episperm of the Kuromane variety of soybeans (this pigment was termed kuromanin [302] before its identity with chrysanthemin was established), and in purple maize.[303] Chrysanthemin is probably identical, moreover, with the anthocyanin of *Rubus fructicosus* (wild and garden blackberry). It has been synthesized from ω-hydroxy-3,4-diacetoxyacetophenone, *o*-tetraacetyl-α-glucosidylbromide and 2-*o*-benzoylphloroglucinol dialdehyde.[304]

Idaein chloride,[305] $C_{21}H_{21}O_{11}Cl$ (3-β-galactosidyl cyanidin chloride, shining brownish-green leaflets or prisms), occurs in *Vaccinium Vitis Idaea*, the skin of red bilberry and in the skins of Jonathan and Stayman Winesap apples.[306] It is identical with the pigment of the leaves of the red beech (*Fagus silvatica*).[307] Idaein chloride has been obtained synthetically from ω-hydroxy-3,4-diacetoxyacetophenone, *o*-tetraacetyl-α-galactosidyl bromide and 2-*o*-benzoylphloroglucinol aldehyde.[308] Idaein has also been obtained from the berries of *Fatsia japonica* where it is accompanied by quercitin.[308a]

Cyanin chloride,[309] $C_{27}H_{31}O_{16}Cl$ (the 3,5-diglucoside of cyanidin chloride, rhombic crystals with a metallic luster), occurs especially in *Rosa gallica*, the red rose, *Centaurea cyanus*, the blue cornflower, and also in the common red poppy.[310] The so-called shisopin [311] found in the leaves of *Perilla ocimoides* L. var. *crispa Beuth.* consists of two components; the one, shisopin A, is identical with cyanin, whereas shisopin B is cyanin esterified with cumaric acid. The constitution of another pigment, perillanin chloride,[312] from the leaves may be susceptible to clarification in a similar sense. Cyanin chloride has been synthesized from *o*-tetraacetyl-β-glucosidyl-phloroglucinol aldehyde and ω-tetraacetyl-β-glucosidoxy-3,4-diacetoxyacetophenone.[313]

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[297] Robertson, Robinson: *J. Chem. Soc.*, **1928**, 1526; Identity of natural and synthetic cyanidin: Robinson, Willstätter: *Ber.*, **61**, 2504 (1928); Kuhn, Wagner-Jauregg: *Ber.*, **61**, 2506 (1928).

[298] Willstätter, Mallison: *Sitzber. Kgl. preuss. Akad. Wiss. (Berlin)*, **1914**, 769.

[299] Willstätter, Bolton: *Ann.*, **412**, 136 (1917); Robinson, Willstätter: *Ber.*, **61**, 2503 (1928); Karrer, Pieper: *Helv. Chim. Acta*, **13**, 1067 (1930).

[300] Hattori, Hayashi: *Acta phytochimica (Japan)*, **10**, 129 (1937).

[301] Hayashi: *Ibid.*, **10**, 139 (1937).

[302] Kuroda, Wada: *Proc. Imp. Acad. Japan*, **9**, 17, 517 (1933); **11**, 189 (1935); *Bull. Chem. Soc. Japan*, **11**, 272 (1936).

[303] Sando, Milner, Sherman: *J. Biol. Chem.*, **109**, 203 (1935).

[304] Robertson, Robinson: *J. Chem. Soc.*, **1927**, 2196; Murakami, Robertson, Robinson: *Ibid.*, **1931**, 2665.

[305] Willstätter, Mallison: *Ann.*, **408**, 15 (1915).

[306] Duncan, Dustman: *J. Am. Chem. Soc.*, **58**, 1511 (1936); Sando: *J. Biol. Chem.*, **117**, 45 (1937).

[307] G. M. Robinson, R. Robinson: *Biochem. J.*, **26**, 1654 (1932).

[308] Robertson, Robinson: *J. Chem. Soc.*, **1927**, 2196; Grove, Robinson: *Ibid.*, **1931**, 2722.

[308a] Hayashi: *Acta Phytochim. (Japan)*, **11**, 91 (1939).

[309] Willstätter, Everest: *Ann.*, **401**, 189 (1913); Willstätter, Nolan: *Ann.*, **408**, 1 (1915).

[310] Schmid, Huber: *Monatsh.*, **57**, 383 (1931); **60**, 285 (1932).

[311] Kuroda: *Proc. Imp. Acad. (Tokyo)*, **11**, 28 (1935); Kuroda, Wada: *Bull. Chem. Soc. Japan*, **11**, 272 (1936).

Mekocyanin chloride,[314] $C_{21}H_{31}O_{16}Cl$ (dark-red rhombododecahedral crystalline granules), according to more recent research,[315,316] is the 3-gentiobioside of cyanidin, and occurs in the flowers of *Papaver Rhoeas* and more particularly in the common red or corn poppy. Partial hydrolysis yields a monoglycoside which is identical with chrysanthemin. Mekocyanin has also been synthesized.[316]

Keracyanin chloride,[317] $C_{27}H_{31}O_{15}Cl$ [yellow-brown prisms or yellow needles (red powder)], is present in the sweet cherry *Prunus avium* and is probably a 3-bioside (see also antirhinin). It breaks down on hydrolysis into cyanidin, glucose and rhamnose.

Prunicyanin chloride,[318] $C_{27}H_{31}O_{15}Cl$ (red flocks which have not yet been completely purified), is present in *Prunus spinosa,* in the skin of the fruit of the sloe tree and probably in *Prunus domestica,* the plum. Both rhamnose and a hexose have been found, and prunicyanin is possibly a 3-bioside.

Sambucin chloride,[319] $C_{27}H_{31}O_{15}Cl$ (red flocks), occurs in *Sambucus nigra,* the black elderberry, and must not be confused with the alkaloid sambucin from the same plant. The anthocyan breaks down into cyanidin, glucose and rhamnose and might appear to be a mixture of mono- and di-glycosides. According to more recent statements,[320] however, elderberries contain two pigments of which one (sambucin) is identical with chrysanthemin whereas the other, $C_{47}H_{50}O_{26}Cl_2$, sambucyanin, appears to be a dimolecular compound of chrysanthemin with a cyanin pentose glycoside in which the sugar group is located in position 3.

Antirhinin chloride,[321] $C_{27}H_{31}O_{15}Cl$, forms bright yellow or scarlet-red needles according to its water of crystallization, and occurs in *Antirhinum majus* in the magenta-colored flowers of snap-dragon, in the flowers of the common flax-weed, *Linaria vulgaris,*[322] and in the flowering currant

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[312] Kondo: *J. Pharm. Soc. (Japan),* **51,** 25 (1931).
[313] Robinson, Todd: *J. Chem. Soc.,* **1932,** 2488; Robinson: *Ber.,* **67A,** 104 (1934).
[314] Willstätter, Weil: *Ann.,* **412,** 231 (1917); Robertson, Robinson: *J. Chem. Soc.,* **1927,** 2196.
[315] G. M. Robinson, R. Robinson: *Nature,* **130,** 21 (1932).
[316] Robinson: *Ber.,* **67A,** 98 (1934); Bell, Robinson: *J. Chem. Soc.,* **1934,** 1604.
[317] Willstätter, Zollinger: *Ann.,* **412,** 164 (1917); Robertson, Robinson: *J. Chem. Soc.,* **1927,** 2196.
[318] Willstätter, Zollinger: *Ann.,* **412,** 164 (1917); Robertson, Robinson: *J. Chem. Soc.,* **1927,** 2196.
[319] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 80 (1927).
[320] Nolan, Casey: *Proc. Roy. Irish Acad. (B),* **40,** 56 (1931).
[321] Scott-Moncrieff: *Biochem. J.,* **24,** 753 (1930).
[322] Hadders, Wehmer in Klein, "Handbuch der Pflanzenanalyse," **III,** 2, p. 986.

Ribes sanguineum.[323] Antirhinin breaks down into cyanidin, rhamnose and glucose and may be a 3-bioside. Both it and sambucin have been thought to be identical with keracyanin, but the researches of other workers have not confirmed the identity.

Peltogynidin,[324] is a new anthocyanidin, closely related to cyanidin and particularly to 8-methylcyanidin, which has been obtained from the leucoanthocyanins of the woods of *Copaifera pubiflora* and various *Peltogynum* species. A 6-hydroxycyanidin [325] has been reported in the beech-acorns of *Fagus silvatica,* in the heart-wood of blackthorn (*Prunus spinosa*) and in the bark and heart-wood of the yew.

Oleocyanin, $C_{27}H_{31}O_5Cl \cdot H_2O$, the olive anthocyanin, is conveniently extracted from the richly pigmented Leccese variety. It is a cyanidin glycoside containing the units of rhamnose and glucose.[325a]

4. Delphinidin chloride,[326] $C_{15}H_{11}O_7Cl$ (3,5,7,3′,4′,5′-hexahydroxyflavylium chloride, brownish-black tablets), decomposes by alkali fission into phloroglucinol, gallic acid, and pyrogallol. Its synthesis [326] has been effected from ω,3,4,5-tetraethoxyacetophenone and 2-hydroxy-4,6-dimethoxybenzaldehyde, and also from ω,3,4,5-tetramethoxyacetophenone and 2-*o*-benzoylphloroglucinol aldehyde.[327]

Pigments Derived from Delphinidin

Gentianin chloride,[328, 329] $C_{30}H_{27}O_{14}Cl$ (blue-red flocks), apparently a monoglycoside, contains one molecule of *p*-hydroxycinnamic acid and occurs in the dwarf gentian, *Gentiana acaulis.*

Vicin chloride,[330] (red-brown needles), apparently a mixture of a monoglucoside and a monorhamnoside, occurs in *Vicia* species. Vicin is only characteristic of the flowers of dark wine-red vetches, as scarlet-red varieties appear to contain other anthocyanins.

Violanin chloride,[331] $C_{36}H_{37}O_{18}Cl$ (blue-violet hexagonal and tetrahedral tablets with a greenish metallic luster) is probably a 3-rhamnoglycoside in which the sugar is esterified with a molecule of *p*-hydroxycinnamic acid. It occurs in the deep blue strains of the garden viola, *Viola tricolor,* which also contain 3-monoglucosides, particularly of delphinidin (this glucoside has been synthesized [332] and undoubtedly occurs naturally) and of peonidin.

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[323] Nolan, Braby: *Proc. Roy. Irish Acad. (B),* **43,** 1 (1936).
[324] G. M. Robinson, R. Robinson: *Biochem. J.,* **27,** 206 (1933).
[325] Willstätter, Mieg: *Ann.,* **408,** 61 (1915).
[325a] Musajo, Minchilli: *Gazz. chim. ital.,* **70,** 293 (1940).
[326] Pratt, Robinson: *J. Chem. Soc.,* **127,** 166 (1925).
[327] Bradley, Robinson, Schwarzenbach: *Ibid.,* **1930,** 793.
[328] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 67 (1927).
[329] *Cf.* Reynolds, Robinson: *J. Chem. Soc.,* **1934,** 1039.
[330] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 67 (1927).
[331] Willstätter, Weil: *Ann.,* **412,** 178 (1917); Karrer, de Meuron: *Helv. Chem. Acta,* **16,** 292 (1933).
[332] Robinson: *Ber.,* **67 A,** 98 (1934).

Hiviscin (hibiscin) chloride,[333] $C_{26}H_{19}O_{16}Cl$ (red-brown needles with a metallic sheen, m.p. 178°), is found in the deep-red calyx of the fruits of *Hibiscus Sabdariffa* accompanied by the pigment gossypetin. It is a delphinidin pentose glycoside containing both glucose and aldopentose.

Hyacin chloride,[334] $C_{27}H_{31}O_{17}Cl$ (blue prismatic needles, m.p. 188° with decomposition), present in *Hyacinthus orientalis* L. (King-of-the-Blues) is probably identical with a delphinidin diglucoside obtained by Robinson [335] from the same plant.

Delphinin chloride,[336] $C_{41}H_{39}O_{21}Cl$ (dark red-brown tablets or prisms), is a diglycoside probably containing two molecules of *p*-hydroxybenzoic acid bound to the sugar residues. It occurs in *Delphinium consolida.*

Delphin chloride,[337] $C_{27}H_{31}O_{17}Cl$, is a 3,5-diglycoside present in the blue flowers of *Salvia patens.* It has been synthesized in small yield from ω-tetraacetyl-β-glucosidoxy-3,4,5-triacetoxyacetophenone and 2-*o*-tetraacetyl-β-glucosidyl-phloroglucinol aldehyde, although the constitution is not regarded by Hayashi [338] as completely established. Delphinidin-3,5-diglucoside forms as awobanin A the chief component of the pigment of Tsuyukusa (*Commelini communis* var. *hortensis* Makino).[339] Awobana is the native name for the flowers of this plant from which the awobana paper of commerce is made. The pigment contains *p*-cumaric acid and a co-pigment, awobanol (pale-yellow needles, m.p. 216°).

Muscadinin, $C_{28}H_{33}O_{17}Cl \cdot 2.5H_2O$, m.p. 184°, occurs in ripe Hunt muscadine grapes. Its color reactions indicate that it is 3:5-diglucosidyl-3′-0-methyl delphinidin chloride.[339a]

Nasunin,[340] the pigment of the egg-plant (Nasu), *Solanum melongena* L. var. *esculentum*, $C_{27}H_{31}O_{17}Cl$, is a delphinidin-3-bioside.

5. Peonidin chloride,[341] $C_{16}H_{13}O_6Cl$ (3,5,7,4′-tetrahydroxy-3′-methoxyflavylium chloride), forms long needles which are reddish grey-brown by transmitted light and breaks down by alkali fission into phloroglucinol and vanillic acid (I):

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[333] Yamamoto, Osima: *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **19**, 134 (1932); *Bull. agri. Chem. Soc. Japan*, **8**, 142 (1932); Sci. Papers, *Inst. Phys. Chem. Research (Tokyo)*, **30**, 258 (1936).

[334] Hayashi: *Acta phytochimica*, **9**, 25 (1936).

[335] G. M. Robinson, R. Robinson: *Biochem. J.*, **26**, 1647 (1932).

[336] Willstätter, Mieg: *Ann.*, **408**, 61 (1915).

[337] Robinson: *Ber.*, **67A**, 100 (1934); Reynolds, Robinson, Scott-Moncrieff: *J. Chem. Soc.*, 1934, 1235.

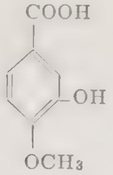
[338] Hayashi: *Acta phytochimica (Japan)*, **9**, 25 (1936).

[339] Kuroda: *Proc. Imp. Acad. (Tokyo)*, **7**, 61 (1931); **9**, 94 (1933); **11**, 238 (1935); *Bull. Chem. Soc. Japan*, **11**, 265 (1936).

[339a] Brown: *J. Am. Chem. Soc.*, **62**, 2808 (1940).

[340] Kuroda, Wada: *Proc. Imp. Acad. (Tokyo)*, **9**, 51 (1933); *Bull. Chem. Soc. Japan*, **11**, 272 (1936).

[341] Willstätter, Nolan: *Ann.*, **408**, 136 (1915); Karrer, Widmer: *Helv. Chim. Acta*, **10**, 8 (1927)



(I)

It has been synthesized from ω-4-diacetoxy-3-methoxyacetophenone and 2,4,6-triacetoxybenzaldehyde[342] and, more satisfactorily from 2-*o*-benzoylphloroglucinol aldehyde and ω-4-diacetoxy-3-methoxyacetophenone.[343]

Pigments Derived from Peonidin

Oxycoccicyanin chloride,[344] $C_{22}H_{23}O_{11}Cl$ (3-β-glucosidylpeonidin chloride, dark-brown needles), occurs in *Vaccinium Vitis Idaea,* the fruit of the bilberry, in *Vaccinium macrocarpum,* the cranberry, and finally in *Oxycoccus macrocarpus,* the American bilberry. The compound was synthesized from ω-tetraacetyl-β-glucosidoxy-4-acetoxy-3-methoxy-acetophenone and 2-*o*-benzoylphloroglucinol aldehyde before its discovery in nature.

Peonin chloride,[345] $C_{28}H_{33}O_{16}Cl$ (reddish-violet needles), is the 3,5-diglucoside occurring in the peony *Paeonia arborea* and also in the red-violet and deep violet flowers of *Pharbitis Nil.*[346] Peonin has been synthesized from ω-*o*-tetraacetyl-β-glucosidoxy-4-acetoxy-3-methoxyacetophenone and 2-*o*-(*o*-tetraacetyl-β-glucosidyl)-phloroglucinol aldehyde.[347]

In garden varieties of peony the varying shades are probably due not to different pigments but only to different amounts of peonin.[347a] Hayashi gives an improved procedure for the isolation of peonin from cultivated varieties of peony.

6. **Petunidin chloride,**[348] $C_{16}H_{13}O_7Cl$ (3,5,7,4′,5′-pentahydroxy-3′-methoxyflavylium chloride, yellowish green prisms), loses one methyl group on heating with hydriodic acid and passes into delphinidin. Petunidin was synthesized from 2-*o*-benzoylphloroglucinol aldehyde and 5-methoxy-3,4-diphenylmethylenedioxyacetophenone,[349] a comparison of the synthetic and natural products confirming almost complete identity, although the latter did not appear to be quite homogeneous.

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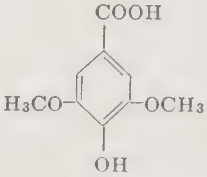
[342] Nolan, Pratt, Robinson: *J. Chem. Soc.*, 1926, 1968.
[343] Murakami, Robinson: *Ibid.*, 1928, 1537.
[344] Levy, Robinson: *Ibid.*, 1931, 2715; Grove, Robinson: *Biochem. J.*, 25, 1706 (1931); *J. Chem. Soc.*, 1931, 2722.
[345] Willstätter, Nolan: *Ann.*, 408, 137 (1915).
[346] Kataoka: *Acta phytochimica (Japan)*, 9, 35 (1936).
[347] Robinson, Todd: *J. Chem. Soc.*, 1932, 2488.
[347a] Hayashi: *Acta phytochim. (Japan)*, 11, 89 (1939).
[348] Willstätter, Burdick: *Ann.*, 412, 217 (1917).
[349] Bradley, Robinson, Schwarzenbach: *J. Chem. Soc.*, 1930, 793; G. M. Robinson, R. Robinson: *Biochem. J.*, 25, 1687 (1931).

Petunin chloride,[348] $C_{28}H_{33}O_{17}Cl$ (rectangular coppery tablets which are violet by transmitted light), is a diglucoside found in the garden petunia *Petunia hybrida.* Both petunidin 3-monoglucoside and 3,5-diglucoside [350] have been prepared.

7. **Malvidin chloride (syringidin chloride),**[351] $C_{17}H_{15}O_7Cl$ (3,5,7,4′-tetrahydroxy-3′5′-dimethoxyflavylium chloride, dark-brown prisms or needles with a bronze luster) undergoes fission by alkali into phloroglucinol and syringic acid (I):



(I)

It has been synthesized [352] from ω-acetoxy-4-benzyloxy-3,5-dimethoxyacetophenone and 2-*o*-benzoylphloroglucinol aldehyde.

Oenin chloride,[353] $C_{23}H_{25}O_{12}Cl$ (red needles), is present in the skin of the black grape and the "Färbertraube" of which the juice is colored. In addition to delphinidin glucosides the chief constituent is 3-β-glucosidylmalvidin chloride which has been synthesized [354] from ω-(*o*-tetraacetyl-β-glucosidoxy)-4-acetoxy-3,5-dimethoxyacetophenone and 2-*o*-benzoylphloroglucinol aldehyde.

Cyclamin,[355] from *Cylamen europaeum,* the cylamen, is identical with oenin, and although the same may be true of the coloring matter [356] of *Primula polyanthus,* the last may be identical with *primulin,*[357] a malvidin-3-galactoside isolated from *Primula sinensis.*

Ampelopsin,[358] the pigment of the skins and flesh of the fruit of the wild vine, *Ampelopsis quinquefolia* Michx., resembles oenin.

Mauretinin chloride,[359] $C_{28}H_{33}O_{17}Cl$, occurs in the bark of the sugar cane (*Mauritius purpur*) and may be hydrolyzed to yield two molecules of glucose and the aglucone mauretinidin chloride, $C_{16}H_{12}O_8Cl$; the latter is a delphinidin monomethyl ether possibly identical with ampelopsidin.

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[350] Robinson: *Ber.,* **67A,** 102 (1934); Bell, Robinson: *J. Chem. Soc.,* **1934,** 1604.

[351] Willstätter, Mieg: *Ann.,* **408,** 122 (1915); Karrer, Widmer: *Helv. Chim. Acta.,* **10,** 5 (1927).

[352] Bradley, Robinson: *J. Chem. Soc.,* **1928,** 1541.

[353] Willstätter, Zollinger: *Ann.,* **408,** 83 (1915); older literature is given here; *Ann.,* **412,** 195 (1917); Anderson, Nabenhauer: *J. Am. Chem. Soc.,* **47,** 2997 (1925); Karrer, Widmer: *Helv. Chim. Acta,* **10,** 5 (1927); Kondo: *J. Pharm. Soc. (Japan),* **50,** 20 (1930).

[354] Levy, Posternack, Robinson: *J. Chem. Soc.,* **1931,** 2701.

[355] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 5 (1927); Bell, Robinson: *J. Chem. Soc.,* **1934,** 813.

[356] Scott-Moncrieff: *Biochem. J.,* **24,** 767 (1930).

[357] Robinson: *Ber.,* **67A,** 98 (1934); Bell, Robinson: *J. Chem. Soc.,* **1934,** 813.

[358] Willstätter, Zollinger: *Ann.,* **412,** 195 (1917); Karrer, Widmer: *Helv. Chim. Acta,* **10,** 5 (1927).

[359] Rao, Walawalkar, Srikantan: *J. Indian Chem. Soc.,* **15,** 27 (1938).

Althaein,[360] the pigment of *Althaea rosea,* the rose mallow or hollyhock, is a mixture of oenin, delphinidin-3′-monomethyl ether and delphinidin itself. Althaein was formerly used in dyeing and printing.

Myrtillin,[361] the coloring matter of the bilberry, *Vaccinium Myrtillus,* is a mixture of the monoglycosides of malvidin and delphinidin, myrtillin *a* and *b* being delphinidin glucoside and galactoside respectively. The controversy relating to the identity or non-identity of the vine and bilberry pigments resulted in a compromise; the ratio of the malvidin to the delphinidin component is different [362] and the bilberry pigments contains an amount of galactose which is lacking in oenin. Whether the position of the sugar residues is different remains to be determined.

Malvin chloride,[363] $C_{29}H_{35}O_{17}Cl$ (dark red-brown needles), is the very widely distributed 3,5-diglucoside. Thus it occurs in the flowers of the wild mallow, *Malva silvestris,* and contaminated by an anthocyan with a lower methoxyl content in the primrose *Primula viscosa* and *Primula integrifolia.* Malvin has been synthesized [364] from 2-*o*-(*o*-tetraacetyl-β-glucosidyl)-phloroglucinol aldehyde and ω-(*o*-tetraacetyl-β-glucosidoxy)-4-acetoxy-3,5-dimethoxyacetophenone.

Negretein chloride,[365] $C_{38}H_{41}O_{18}Cl$, is found with tuberin chloride in the violet-colored potato and undergoes fission into *p*-hydrocinnamic acid, malvidin chloride, glucose and isorhodeose, a methylpentose.

Tuberin chloride, $C_{22}H_{23}O_{12}Cl$, is a 3-monoglucoside which may be hydrolyzed to glucose and tuberidin chloride, $C_{16}H_{13}O_7Cl$, an aglucone containing one methoxyl and five hydroxyl groups and yielding *p*-hydrobenzoic acid on fission.

Ensatin, $C_{38}H_{41}O_{19}Cl \cdot 10H_2O$, m.p. 175° (decomp.), is a pigment which has been isolated from *Iris ensata* Thunberg var. *hortensis* Makino et Nemoto. On hydroylsis it first loses one molecule of *p*-hydroxycinnamic acid with formation of *isomalvan* chloride, $C_{29}H_{35}O_{17} \cdot 8H_2O$, m.p. 165°; the latter then decomposes into glucose and malvidin. Ensatin then is regarded as a malvidin-3-bioside with *p*-hydroxycinnamic acid linked to the sugar residue.[365a]

8. **Hirsutidin chloride,**[366] $C_{18}H_{17}O_7Cl$ (3,5,4′-trihydroxy-7,3′,5′-trimethoxyflavylium chloride, dark-red prisms), has been synthesized from

[360] Willstätter, Martin: *Ann.*, **408,** 110 (1915); Karrer, Widmer; *Helv. Chim. Acta,* **10,** 5 (1927); Kondo: *J. Pharm. Soc. (Japan),* **50,** 19 (1930); Karrer, Weber: *Helv. Chim. Acta,* **19,** 1025 (1936).

[361] Willstätter, Zollinger: *Ann.*, **408,** 83 (1915); **412,** 195 (1917); Karrer, Widmer: *Helv. Chim. Acta,* **10,** 5 (1927); Karrer: *Collegium,* **1931,** 700; Bell, Robinson: *J. Chem. Soc.*, **1934,** 813; also petuninglycoside; Reynolds, Robinson: *J. Chem. Soc.*, **1934,** 1039.

[362] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 5 (1927).

[363] Willstätter, Mieg: *Ann.*, **408,** 122 (1915); Karrer, Widmer: *Helv. Chim. Acta,* **10,** 14, 758 (1927).

[364] Robinson, Todd: *J. Chem. Soc.*, **1932,** 2299.

[365] Chmielewska: *Roczniki Chemji,* **15,** 491 (1935); *Chem. Zentr.*, **1936,** I, 2361.

[365a] Hayashi, *Proc. Imp. Acad. (Tokyo),* **16,** 478 (1940).

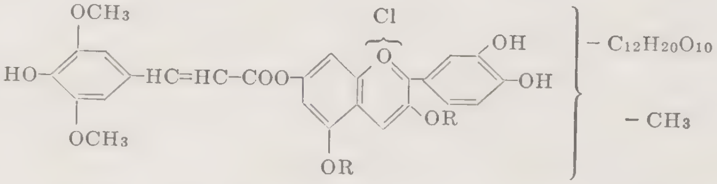
[366] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 758 (1927); Bradley, Robinson, Schwarzenbach: *J. Chem. Soc.*, **1930,** 793.

the 4-methyl ether of 2-*o*-benzoylphloroglucinol aldehyde and ω-acetoxy-4-benzoyloxy-3,5-dimethoxyacetophenone.

Pigments Derived from Hirsutidin

Hirsutin chloride,[367] $C_{30}H_{37}O_{17}Cl$ (red-brown needles, the 3,5-diglucoside), is found in the flowers of *Primula hirsuta.* Oxidation yielded a hirsutone analogous to malvone, from which syringic acid was obtained by alkali fission. A synthesis [368] was effected from 2-*o*-(*o*-tetraacetyl-β-glucosidoxy)-4-*o*-methylphloroglucinol aldehyde and ω-(*o*-tetraacetyl-β-glucosidoxy)-4-acetoxy-3,5-dimethoxyacetophenone.

The pigment of the red cabbage, *Brassica oleracea,*[369] *rubrobrassicin chloride,* $C_{28}H_{33}O_{16}Cl$, is assigned the following constitution:



The sugar and methyl residues are to be included in the groups R and R′. The pigment, it will be noted, is an ester of synapinic acid.

Acridioxanthin. This is a pigment of anthocyanin character which has been isolated from a number of orthoptera. Though its chemistry awaits fuller investigation, it is interesting, since a similar and possibly identical anthocyanin occurs in the plants on which the insects live, so that it is suggested that acridioxanthin finds its origin in the ingested foodstuffs.[369a]

Betanin,[370] $C_{21}H_{23}O_{10}N_2Cl$ (?) is a nitrogenous anthocyan obtained from the red beet, *Beta vulgaris.* It forms glistening bronze-green crystals. Betanin may be degraded to glucose and betanidin, $C_{20}H_{23}O_7N_2Cl$, amorphous purple material with a green sheen which is very sensitive toward oxygen. It contains no methoxyl groups or N-methyl groups but takes up two methyl groups on methylation. Fusion with alkali gave a pyrrole derivative and small amounts of butyric or valeric acid, but no phloroglucinol. It is suggested that betanidin is a pentahydroxyflavylium derivative containing an ornithin system with the strongly basic nitrogen adjacent to the benzene ring.

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[367] Karrer, Widmer: *Helv. Chim. Acta,* **10,** 758 (1927).

[368] Robinson, Todd: *J. Chem. Soc.,* **1932,** 2293; syntheses of 5-β-glucosidyl- and 5-lactosidylhirsutidin chloride: Levy, Robinson: *J. Chem. Soc.,* **1931,** 2738; of 3-β-glucosidyl-hirsutidin chloride: Levy, Posternack, Robinson: *J. Chem. Soc.,* **1931,** 2701.

[369] Willstaedt: *Biochem. Z.,* **242,** 303 (1931); **276,** 262 (1935); Chmielewska: *Roczniki Chemji* ,**13,** 725 (1933); **16,** 384 (1936).

[369a] Chauvin, *Compt. rend.,* **211,** 339 (1940).

[370] Schudel: *Dissertation,* Zurich, 1918 (carried out with Willstätter); G. M. Robinson, R. Robinson: *J. Chem. Soc.,* **1932,** 1439; **1933,** 25; Ainley, Robinson: *J. Chem. Soc.,* **1937,** 446: Schmidt: *Naturwiss.,* **25,** 284 (1937); synthetic experiments: Ainley, Robinson: *J. Chem. Soc.,* 1937, 453: Chmielewska: *Roczniki Chemji,* **18,** 1 (1938); *Chem. Zentr.,* **1939,** I, 1370; Pucher, Curtis, Vickery: *J. Biol. Chem.,* **123,** 61, 71 (1938).

Similar pigments have been found in *Celosia plumosa, Celosia cristata* and in *Atriplex hortensis atrosangineus,* winter spinach.* The pigment *bougainvillaeidin chloride*[371] (black needles), $C_{22}H_{26\text{-}30}O_{10}NCl$, from *Bougainvillaea glabra,* is possibly a N-methylated condensation product of leucin and delphinidin chloride or an isomeride thereof. The episperm of the seeds of *Abrus precatorius*[372] contains a glucoside which may be hydrolyzed to glucose and abranidin, a dark-red granular powder. Finally it must be mentioned that anthocyans have also been detected in ferns.[373] Thus *Davallia divaricata* contains mixed pelargonidin (and cyanidin) diglycosides in an acylated form, and anthocyans of types not previously encountered have been found in *Osmunda regalis* var. *Hillii, Dryopteris varia, Adiantum Vietchianum* and *Polypodium rhodoleuron.*

Pigments of Red- (Brazil-) and Log-wood

These pigments[374] comprise brazilein and haematein obtained by oxidation of brazilin occurring in red- or brazil-wood, and haematoxylin[375] from logwood.

Brazilein.[376] Redwood, or more generally, soluble redwoods are the products of trees belonging (as do those producing logwood described below) to the family of *Leguminosae* (*Caesalpiniaceae*) and growing in the East Indies, South and Central America and in Africa. The term brazil-wood is derived from the word *braza,* signifying "glowing like fire." Among the woods which come into consideration there are distinguished: Pernambuco or Fernambuco wood from *Caesalpinia crista,* bahia or brazil-wood proper from *Caesalpinia brasiliensis,* lima wood (resembling St. Martha and Nicaragua redwoods) from *Caesalpinia echinata,* and finally sappan wood from *Caesalpinia sapan,* all these woods being of a bright red color. Brazilin[377] does not appear to exist in redwood as a glycoside.

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* Robinson has suggested that the whole group of coloring matters resembling anthocyanins should be termed flavocyanins. Nudicaulin, $C_{30}H_{38}O_{15}NCl$ (chloride), obtained from *Papaver nudicaule,* appears to contain one amino group.[370a]

[370a] Robinson, Price: *J. Chem. Soc.,* **1939,** 1465.

[371] Price, Robinson: *J. Chem. Soc.,* **1937,** 449.

[372] Ghatek: *Bull. Acad. Sci. of United Provinces of Agra and Oudh (Allabahad),* **3,** 69 (1933); *Chem. Zentr.,* **1935,** I, 576.

[373] Price, Sturgess, R. Robinson, G. Robinson: *Nature,* **142,** 356 (1938).

[374] For earlier accounts of these pigments see Rupe: "Die Chemie der natürlichen Farbstoffe," Vol. **I,** p. 103; Vol. **II,** p. 173.

[375] The name haematin is not used, to avoid confusion with the blood pigments.

[376] More recent account of the occurrence of brazil-wood: Mell: *Textile Col.,* **51,** 820 (1929); For methods of working up redwood see Ullmann: "Encyclopädie der technischen Chemie," 2nd ed., Vol. **5,** p. 143.

[377] Ullmann: "Encyclopädie der technischen Chemie," Vol. **5,** p. 144.

Constitution of brazilin: Brazilin was first isolated from brazil-wood by Chevreul[378] as colorless silky needles to which the empirical molecular formula $C_{16}H_{14}O_5$ was assigned.[379] A first glimpse into the constitution of brazilin was obtained when the presence of four hydroxyl groups was established by acetylation. One of these hydroxyl groups may be distinguished from the others in that methylation[380] with methyl iodide gives only a trimethyl ether, which may on the other hand yield an acetyltrimethylether, whereas more drastic conditions must be employed to obtain a tetramethyl derivative. From this observation it was concluded that this behavior is due to the presence of an alcoholic hydroxyl group. Either dry distillation[381] or the action of fused caustic potash[382] yielded resorcinol; and by a somewhat modified procedure protocatechuic acid[383] was obtained. Finally it was quite early demonstrated that cautious oxidation of brazilin by a variety of methods[384] afforded the pigment brazilein, according to the equation:

$$C_{16}H_{14}O_5 + O = C_{16}H_{12}O_5 + H_2O$$

Brazilein forms silvery leaflets which dissolve in water to a rose-red solution with an orange fluorescence.

The oxidation of alkaline brazilin solutions under milder conditions, *e.g.*, by the action of atmospheric oxygen, leads to the formation of brazilein, but the prolonged action of oxygen results in the breakdown of the brazilin molecule. Among the oxidation products[385] may be recognized β-resorcylic acid (2,4-dihydroxybenzene-1-carboxylic acid), and on the other hand a compound of the composition $C_9H_6O_4$ (I) which contains two hydroxyl groups. This last fragment yields a dimethyl ether (II) which undergoes further oxidation to 4-methoxy-2-hydroxybenzoic acid, and on boiling with alcoholic sodium hydroxide undergoes fission to formic acid and fisetol dimethyl ether (III):

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[378] Chevreul: *Ann. chim. (1)*, **66**, 225 (1808).

[379] Liebermann, Burg: *Ber.*, **9**, 1883 (1876).

[380] Alkyl derivatives of brazilin: Dralle: *Ber.*, **17**, 375 (1884); Schall, Dralle: *Ber.*, **20**, 3365 (1887); **21**, 3009 (1888); **22**, 1547 (1889); **23**, 1428 (1890); Schall: *Ber.*, **25**, 3670 (1892); **27**, 524 (1894); Herzig: *Monatsh.*, **14**, 56 (1893); **15**, 139 (1894); **16**, 906 (1895); **19**, 738 (1898); Herzig, Pollak: *Monatsh.*, **23**, 165 (1902); Gilbody, W. H. Perkin, Jr., Yates: *J. Chem. Soc.*, **79**, 1396 (1901); v. Kostanecki, Tambor: *Ber.*, **35**, 1867 (1902); Zerewitinoff: *Ber.*, **41**, 2233 (1908).

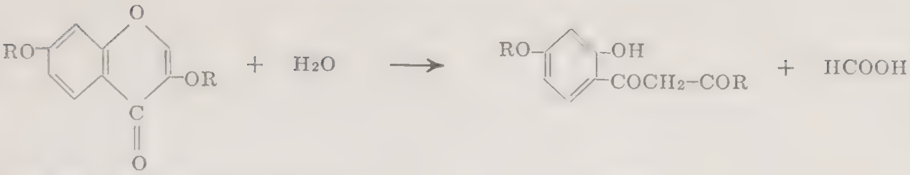
[381] Kopp: *Ber.*, **6**, 446 (1873).

[382] Liebermann, Burg: *Ber.*, **9**, 1883 (1876); Wiedemann: *Ber.*, **17**, 194 (1884); Dralle: *Ber.*, **17**, 375 (1884).

[383] Herzig: *Monatsh.*, **29**, 739 (1908).

[384] Reim: *Ber.*, **4**, 329 (1871); Liebermann, Burg: *Ber.*, **9**, 1886 (1876); Buchka, Erck: *Ber.*, **18**, 1138 (1885); Hummel, A. G. Perkin: *J. Chem. Soc.*, **41**, 367 (1882); *Ber.*, **15**, 2337 (1882); Schall, Dralle: *Ber.*, **23**, 1428 (1890); action of acids: Bolley: *Schweiz. polytech. Ztg.*, **9**, 267 (1864); Schall: *Ber.*, **27**, 524 (1894); Dralle: *Ber.*, **17**, 375 (1884).

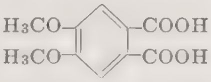
[385] Schall, Dralle: *Ber.*, **21**, 3009 (1888); **22**, 1547 (1889); **25**, 18 (1892); Schall: *Ber.*, **27**, 524 (1894); **32**, 1045 (1899); Feuerstein, v. Kostanecki: *Ber.*, **32**, 1024 (1899); Bloch, v. Kostanecki: *Ber.*, **33**, 473, note (1900); Pfeiffer, Oberlin: *Ber.*, **57**, 208 (1924); Pfeiffer, Oberlin, Konermann: *Ber.*, **58**, 1947 (1925).



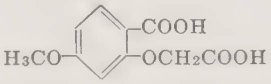
(I) R = H, 3,7-dihydroxychromone (III)
(II) R = CH_3, Dimethyl ether

(I) is thus 3,7-dihydroxychromone which was obtained synthetically.[386] It should be stated here that the constitutional formulas originally proposed on the basis of these facts have not been substantiated by the results of further research.

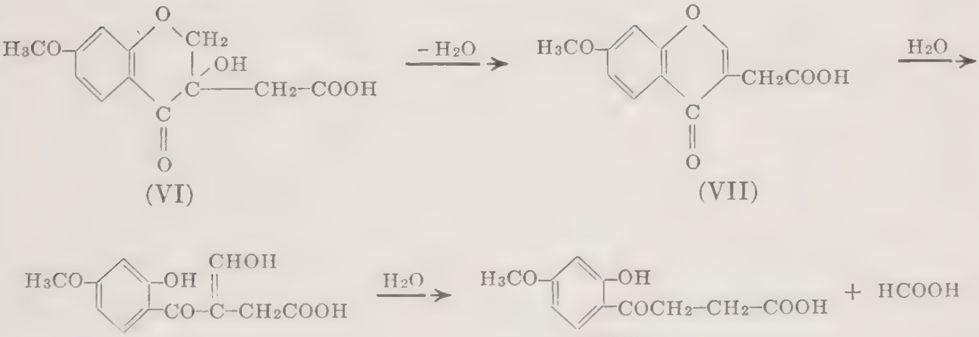
Thus later work[387] has demonstrated that the following fragments may be obtained by oxidizing trimethylbrazilin with potassium permanganate; first, *m*-hemipinic acid (IV), then 1-carboxy-4-methoxy-2-phenoxyacetic acid (V), the constitution of which was established by partial synthesis and related considerations, and finally brazilic acid (VI)



(IV)



(V)



(VI) (VII)

(VIII)

The constitution of brazilic acid[388] follows from the fact that, on being warmed with concentrated sulfuric acid, it yields anhydrobrazilic acid (VII); this is 7-methoxychromone-3-acetic acid, as it is split by baryta water into β-(2-hydroxy-4-methoxy-benzoyl)-propionic acid (VIII), and formic acid and its ester may be resynthesized from the ester of (VIII) and formic ester. It may thus logically be deduced that of the above oxidation products of trimethylbrazilin, β-resorcylic and carboxymethoxyphenoxyacetic acids are fission products of brazilic acid, whereas

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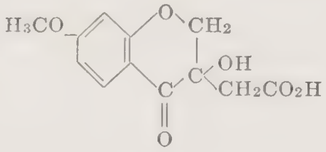
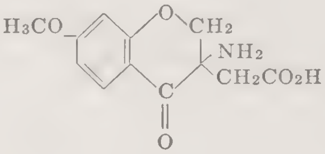
[386] Pfeiffer, Oberlin, Konermann: *Ber.*, 58, 1947 (1925).

[387] Gilbody, W. H. Perkin, Jr., *Proc. Chem. Soc. London*, 15, 241 (1899); Gilbody, W. H. Perkin, Jr., Yates: *J. Chem. Soc.*, 79, 1396 (1901).

m-hemipinic acid has its origin in the second half of the brazilin molecule. Brazilic acid, the only degradation product of brazilin to contain the characteristic hydroxyl group, has been synthesized:

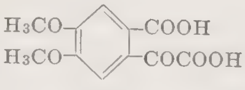


Brazilic acid

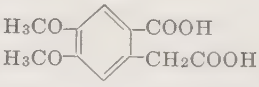


(A)

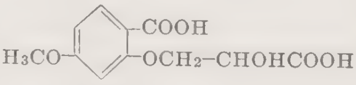
The *d*-, *l*-, and inactive forms of an intermediate amino-acid were each converted into the corresponding stereoisomerides of the aminochromanone acetic acid (A), but all these yielded only inactive brazilic acid.[387a] The complete degradation of trimethylbrazilin by a different route [389] through trimethylbrazilone (see below) gave, in addition to *m*-hemipinic acid and carboxymethoxyphenoxyacetic acid, three other acids, 4,5-dimethoxy-1-carboxy-2-benzoylformic (I),[390] 4,5-dimethoxy-1-carboxy-2-phenylacetic (II), and 4-methoxy-1-carboxy-2-phenoxylacetic (III) acids:



(I)



(II)



(III)

Once again one may assume that the monomethoxy derivatives originate in one half of the molecule and the dimethoxy compounds in the other half.

A more illuminating glimpse is provided by a larger fragment obtained by oxidizing trimethylbrazilin with potassium permanganate. This is brazilinic acid (IV), an acid of molecular formula $C_{19}H_{18}O_9$, the constitution of which was established by its synthesis [391] from ethyl methoxyphenoxyacetate and hemipinic anhydride:

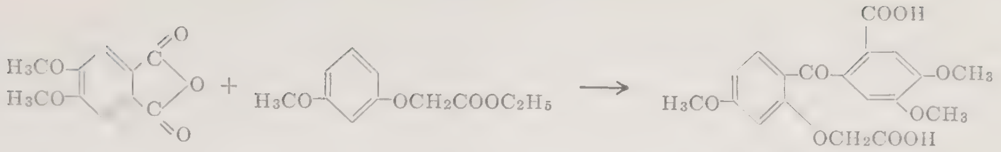
[387a] Pfeiffer, Heinrich, *J. prakt. Chem.*, **156**, 241 (1940). For further "model" synthetic work see Pfeiffer, Epler, *Annalen*, **545**, 263 (1940).

[388] Gilbody, W. H. Perkin, Jr., Yates: *J. Chem. Soc.*, **79**, 1396 (1901); W. H. Perkin, Jr.: *J. Chem. Soc.*, **81**, 221 (1008 (1902); *Ber.*, **35**, 2946 (1902); W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **93**, 489 (1908).

[389] Gilbody, W. H. Perkin, Jr.: *Proc. Chem. Soc. London*, **15**, 27 (1899); Gilbody, W. H. Perkin, Jr., Yates: *Ibid.*, **16**, 105 (1900); W. H. Perkin, Jr.: *J. Chem. Soc.*, **81**, 1008 (1902).

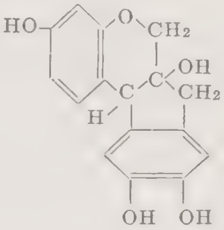
[390] Synthesis: Harding, Weizmann: *J. Chem. Soc.*, **97**, 1126 (1910).

[391] Further synthesis: Rây, Silooja, Wadha: *J. Indian Chem. Soc.*, **10**, 617 (1933).

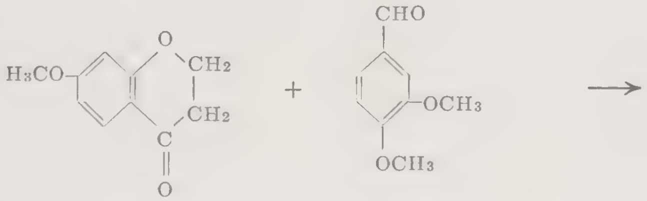


(IV)

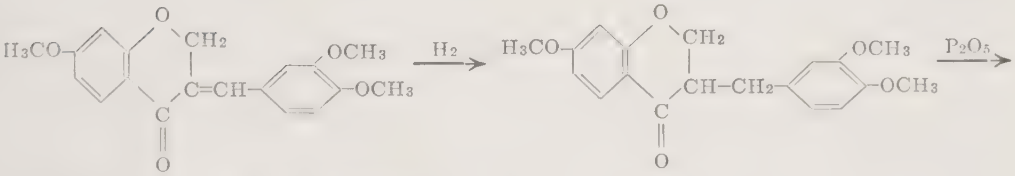
Taking account of the molecular formula and the constitution of the degradation products and piecing together all the above facts, the following constitution for brazilin was proposed by Pfeiffer[392]:



This was substantiated by the work of W. H. Perkin, Jr.,[393] and justified synthetically as far as the location of the alcoholic hydroxyl group by the synthesis of trimethylbrazilone by Pfeiffer[394]:



7-Methoxychromanone *Vanillin methyl ether*



3,4-Dimethoxybenzalmethoxychromane

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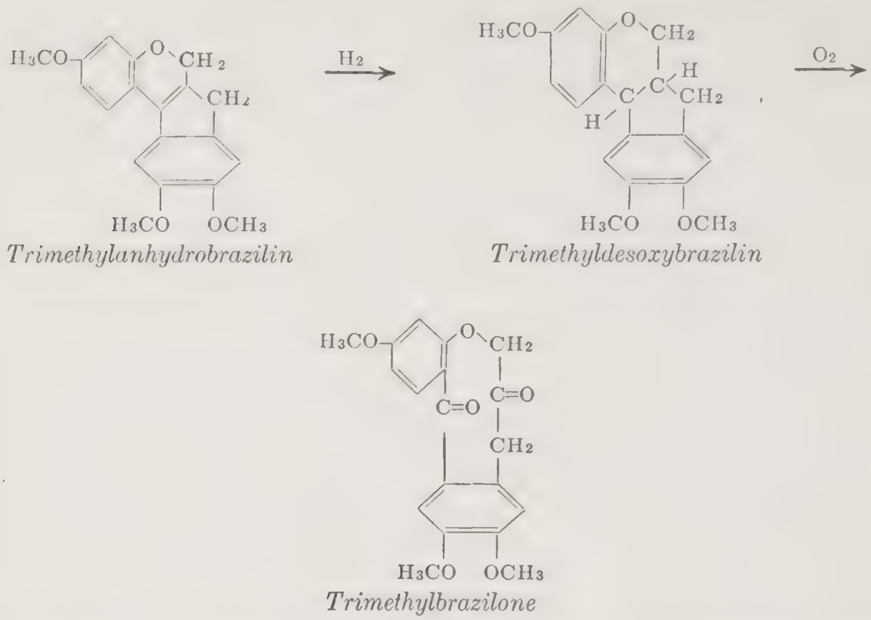
[392] Werner, Pfeiffer: *Chem. Z.*, **3**, 388, 420 (1904).

[393] W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **93**, 489 (1908); older brazilin formulas: v. Kostanecki: *Z. Farben Textilchem.*, **3**, 14 (1904); Herzig: *Monatsh.*, **20**, 461 (1899); Herzig, Pollak: *Monatsh.*, **22**, 207 (1901); **25**, 871 (1904); **27**, 743 (1906).

[394] Pfeiffer, Angern, Haack, Willems: *Ber.*, **61**, 839 (1928); Pfeiffer, Willems: *Ber.*, **62**, 1242 (1929); Pfeiffer, Quehl, Tappermann: *Ber.*, **63**, 1301 (1930); Pfeiffer, Hilpert, Schneider: *J. prakt. Chem. (2)*, **137**, 227 (1933); Pfeiffer, Schneider: *J. prakt. Chem. (2)*, **140**, 9 (1934); cf. synthetic experiments: W. H. Perkin, Jr., Robinson: *Proc. Chem. Soc. London*, **22**, 160 (1906); Pfeiffer, Grimmer: *Ber.*, **50**, 911 (1917); Pfeiffer, Emmer: *Ber.*, **53**, 945 (1920); Pfeiffer, Oberlin, Konermann: *Ber.*, **58**, 1947 (1925); cf. also Crabtree, Robinson: *J. Chem. Soc.*, **113**, 859 (note) (1918); W. H. Perkin, Jr., Rây, Robinson: *J. Chem. Soc.*, **1926**, 941; **1928**, 1504; Pfeiffer, Angern, Haack, Willems: *Ber.*, **61**, 1923 (1928); Appel, Baker, Hagenbach, Robinson: *J. Chem. Soc.*, **1937**, 738; Pfeiffer, Döring, Kobs, Werner: *J. prakt. Chem. (2)*, **150**, 199 (1938); Pfeiffer, Döring: *Ber.*, **71**, 279 (1938).

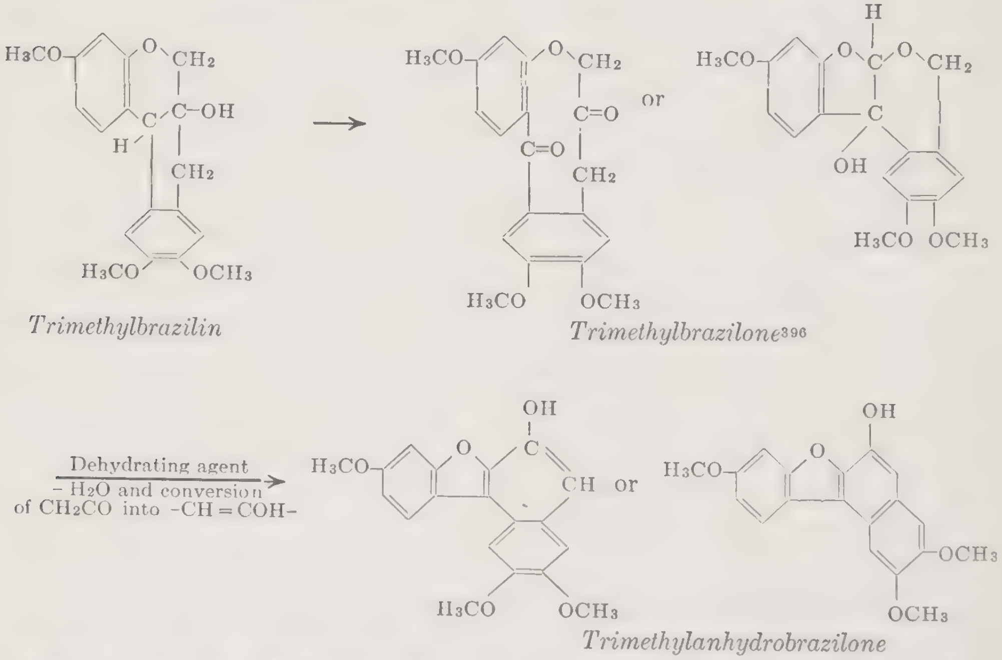


Trimethylanhydrobrazilin

Trimethyldesoxybrazilin

Trimethylbrazilone

On the basis of these reactions the oxidation of trimethylbrazilin with chromic acid follows the course[395]:



Trimethylbrazilin

Trimethylbrazilone[396]

Trimethylanhydrobrazilone

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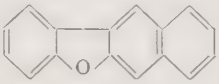
[395] Herzig, Pollak: *Ber.*, **36**, 398, 1220 (1903); *Monatsh.*, **23**, 165 (1902); **25**, 871 (1904); *Ber.*, **38**, 2166 (1905); W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **93**, 489 (1908); Engels, W. H. Perkin, Jr., Robinson: *Ibid.*, **93**, 1115 (1908); pseudotrimethylbrazilone, a conversion product of trimethylbrazilone: Herzig, Pollak: *Ber.*, **37**, 631 (1904); Monatsh., **27**, 743 (1906); nitrogenous derivatives of trimethylbrazilone: Herzig, Pollak: *Ber.*, **36**, 2319, 3713 (1903); **38**, 2166 (1905); W. H. Perkin, Jr.: *Proc. Chem. Soc. London*, **18**, 147 (1902); Gilbody, W. H. Perkin, Jr.: *J. Chem. Soc.*, **81**, 1040 (1902); W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **95**, 381 (1909).

[396] Pfeiffer, Oberlin: [*Ber.*, **60**, 2142 (1927)] and W. H. Perkin, Jr., Rây, Robinson [*J. Chem. Soc.*, **1927**, 2094] now regard the first formula for trimethylbrazilone as the more probable.

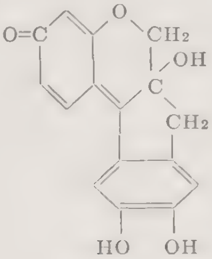
Trimethylanhydrobrazilone is thus a derivative of β-naphthol and accordingly dissolves in alkali and couples with diazo-compounds.

Some contribution to the research on the constitution of brazilin was due to v. Kostanecki,[397] although the various structural formulas proposed by him [398] were not acceptable. v. Kostanecki's work included, however, his observation that treatment of trimethylbrazilin with hydriodic acid yielded a compound [399] with the empirical molecular formula $C_{10}H_7O(OH)_3$. On distillation of this new compound with zinc dust a so-called brazane (I) was obtained:

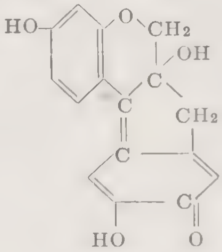


(I)

and although the constitution assigned was confirmed by synthesis, the formation of brazane is not easily reconciled with the accepted brazilin formula.[400] No rational degradation is yet known in which the indane skeleton is retained. The formula proposed by Herzig [401] has likewise been rejected. Admitting now that the structure of brazilin has been established with almost complete certainty, the constitution of brazilein may be deduced, remembering that it contains two hydrogen atoms fewer than the parent brazilin. This difference is best expressed by the assumption of a quinonoid formula [402] when two possibilities present themselves, (II) and (III).



(*Pfeiffer*)
(II)



(*Perkin*)
(III)

Preference is given to formula (III) of Perkin,[403] as it would be in accordance with the fact that oxidation of trimethyldesoxybrazilin results in oxidative attack on the pyrocatechol residue.

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[397] cf. Feuerstein, v. Kostanecki: *Ber.*, 32, 1024 (1899).

[398] v. Kostanecki, Lampe: *Ber.*, 35, 1667 (1902).

[399] v. Kostanecki, Lloyd: *Ber.*, 36, 2193, 2199 (1903); cf. Bollina, v. Kostanecki, Tambor: *Ber.*, 35, 1675 (1902); v. Kostanecki, Paul: *Ber.*, 35, 2608 (1902); synthesis of brazane: v. Kostanecki, Lampe: *Ber.*, 41, 2373 (1908).

[400] W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, 95, 381 (1909).

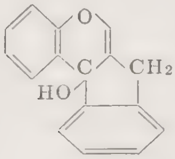
[401] Herzig, Pollak: *Ber.*, 39, 265 (1906); *Monatsh.*, 27, 743 (1906); for Herzig's contribution to the formula of brazilin, see Pollak: (obituary to Herzig) *Ber.*, **58A**, 69 (1925).

[402] cf. Herzig: *Chemiker. Ztg.*, 27, 202 (1903); *Ber.*, 36, 3951 (1903).

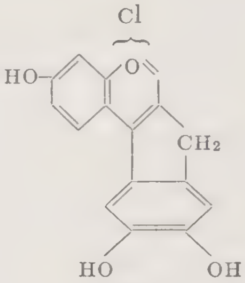
[403] Pfeiffer, Schneider: *J. prakt. Chem. (2)*, 144, 54 (1936); Mićović, Robinson: *J. Chem. Soc.*, 1937, 43.

The reconversion of brazilein into brazilin may also be effected. Attention may be drawn to the fact that comparing the anthoxanthidins and anthocyanidins, the phenyl group in brazilein is located on carbon atom 4 of the benzpyrane residue, instead of in position 2 or, as in the case of isoflavone derivatives, in position 3.

If brazilein is dissolved in sulfuric acid and the solution then precipitated with acetic acid, an isobrazilein sulfate is obtained; similarly, treatment of brazilein with hydrochloric acid yields isobrazilein chloride [404] from which an isobrazilein of empirical formula $C_{16}H_{12}O_5$ may be obtained by treating with silver oxide. The isobrazilein compounds (V) are derived from 3,4-indenobenzpyranol [405] (IV) and thus stand in the same relation to brazilein as anthocyanidin does to anthoxanthidin:

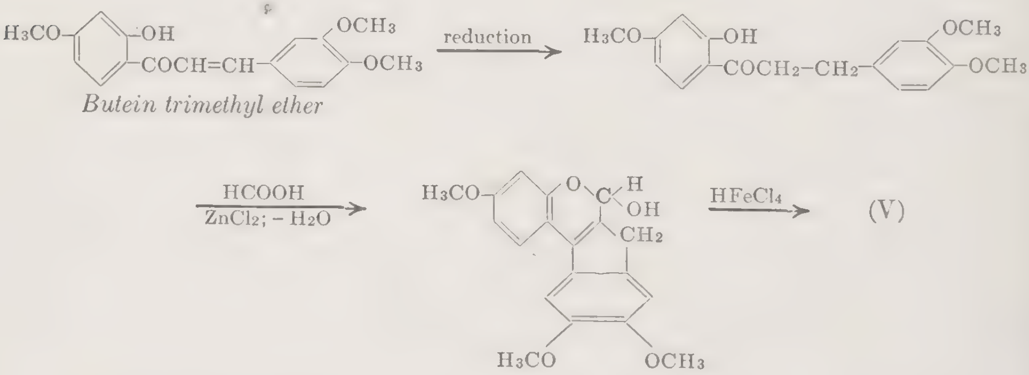


(IV)


Cl
HO
O
CH2
HO
OH

(V)

It has recently been proposed [406] that isobrazilein should be described as anhydrobrazilein. Isobrazilein chloride [407] has been synthesized as shown schematically below:


H3CO
OH
COCH=CH
OCH3
OCH3
Butein trimethyl ether
reduction
H3CO
OH
COCH2–CH2
OCH3
OCH3
HCOOH
ZnCl2; – H2O
H3CO
O
C
H
OH
CH2
H3CO
OCH3
HFeCl4
(V)

The relationship between the more important derivatives of brazilin are shown on pages 242 and 243.

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[404] Hummel, A. G. Perkin: *J. Chem. Soc.*, **41**, 367 (1882).
[405] Engels, W. H. Perkin, Jr., Robinson: *Ibid.*, **93**, 1115 (1908).
[406] Mićović, Robinson: *Ibid.*, **1937**, 43.
[407] Crabtree, Robinson: *Ibid.*, **113**, 859 (1918).

Brazilin is best obtained by ethereal extraction of the commercial extract prepared by lixiviation of the finely divided wood. Brazilein may be prepared by adding excess of ammonia to an aqueous extract of brazilwood and leading air through the solution.

Uses of brazil-wood extracts: The extracts find application at the present day only in cotton printing; much less is used in cotton dyeing, and the dyeing of wool and silk with redwood colors has practically ceased. The cotton is mordanted with a tanning agent, *e.g.*, sumach, and alum, tin or iron salts, etc., and the dyestuff extracts [408] are conveniently allowed to ferment before use, so that brazilin is converted into brazilein. Soft bluish-red dyeings are the result if aluminum is the mordant, and tin gives orange and iron violet-grey shades; the dyeings are, however, rather fugitive to light, chlorine, soap and acids.

Haematein. Logwood,[409] or campeachy wood, is obtained from *Haematoxylon campechianum,* a tree of the *Caesalpiniaceae* family growing in Central America and the West Indies. The wood was introduced into Europe shortly after the discovery of America, individual varieties, all of them of a dark color, being known and distinguished according to the port of shipment.

Constitution of haematein [410]: Haematoxylin may be obtained from logwood in the crystalline condition [411] and forms colorless needles with the empirical molecular formula [412] $C_{16}H_{14}O_6$. It takes up five acetyl groups [413] and therefore contains five hydroxyl groups of which one is alcoholic in character.[414] It was quite early postulated that brazilin and haematoxylin [415] possess closely related constitutions, when the extra oxygen atom in the haematoxylin formula would be accounted for by an additional hydroxyl group. Support for this supposition was found in the fact that, on fusion with potash, haematoxylin yielded pyrogallol [416] instead of the resorcinol obtained from brazilin. Moreover, the oxidation [417] of tetramethylhaematoxylin with potassium permanganate to

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[408] Assay of extracts: v. Cochenhausen: *Angew. Chem.*, **17**, 877 (1904); Tinctorial properties of isobrazilein: d'Andiran: *Bull. Soc. Ind. Mulhouse*, **75**, 385 (1905).

[409] Ullmann: "Encyclopädie der technischen Chemie," 2nd ed., Vol. **5**, p. 116; cf. also Mell [*Textile Col.*, **51**, 257 (1929)] on a logwood coloring matter in African camwood; also Mell [*Textile Col.*, 53, 254, 337, 402 (1931)], a historical study.

[410] As the determination of the constitution of brazilein went hand in hand with that of haematein, almost all the references given under brazilein are also of significance here. The most important are repeated once more and those dealing specifically with haematein are included. Among earlier reviews, apart from those quoted under brazilein, are v. Kostanecki: *Z. Farben. Textilchemie*, **3**, 4 (1904), also Rost: "Monographie des Haematoxylin," Bern, 1904.

[411] Chevreul: *Ann. chim. (1)*, **81**, 128 (1812); **82**, 53 (1812).

[412] O. L. Erdmann: *Ann.*, **44**, 292 (1842); *J. prakt. Chem.*, **26**, 199 (1842); **75**, 218 (1858); Hesse: *Ann.*, **109**, 332 (1858).

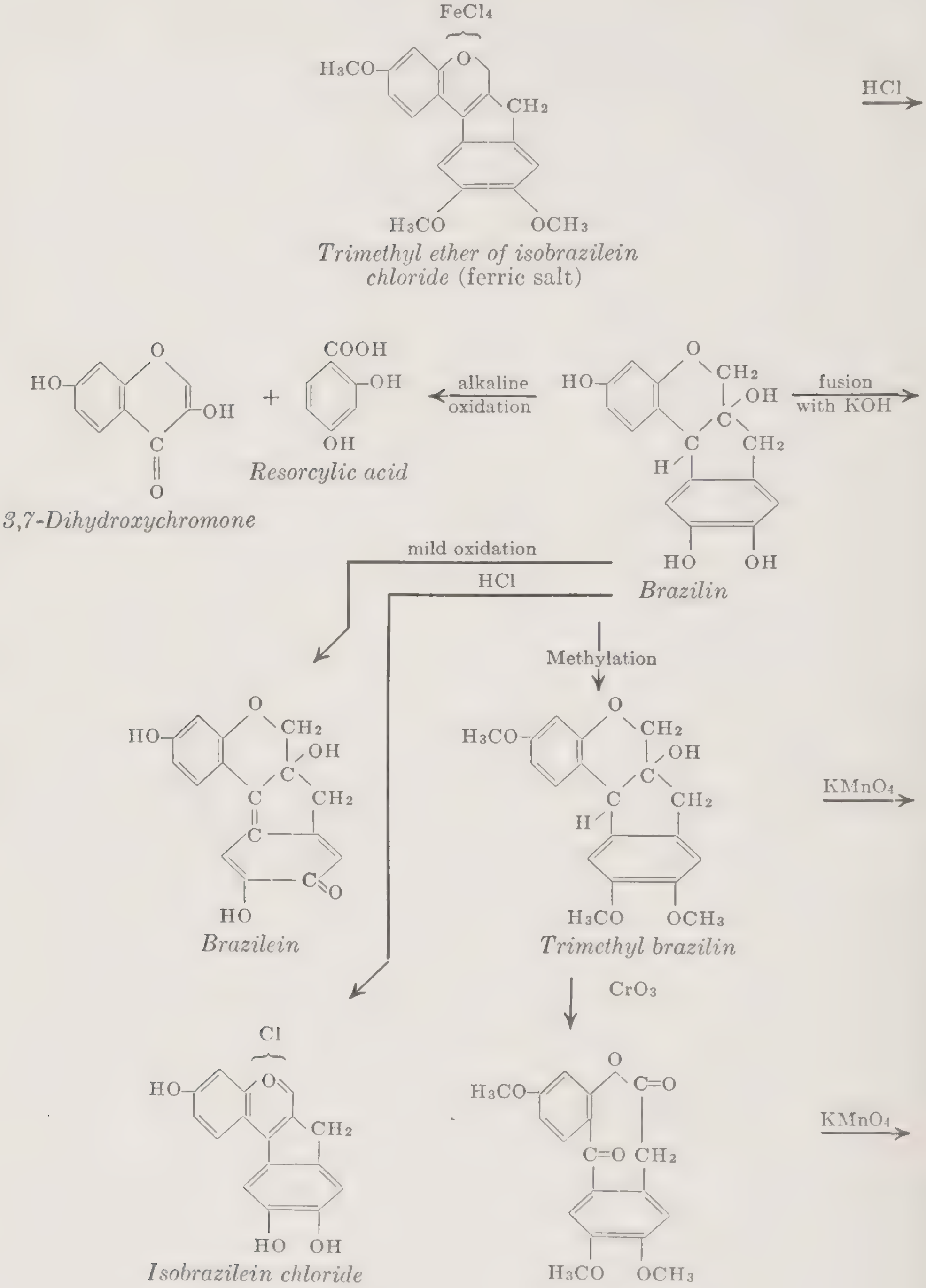
[413] E. Erdmann, Schultz: *Ann.*, **216**, 232 (1883).

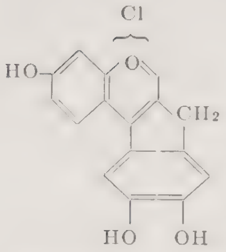
[414] Herzig: *Monatsh.*, **15**, 139 (1894).

[415] Liebermann, Burg: *Ber.*, **9**, 1883 (1876); Hummel, A. G. Perkin: *J. Chem. Soc.*, **41**, 367 (1882).

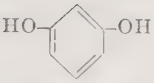
[416] Reim: *Ber.*, **4**, 329 (1871); R. Meyer: *Ber.*, **12**, 1392 (1879).

[417] W. H. Perkin, Jr., *J. Chem. Soc.*, **81**, 1057 (1902).


FeCl₄
H₃CO
CH₂
H₃CO
OCH₃
Trimethyl ether of isobrazilein chloride (ferric salt)
HCl
HO
O
OH
C
O
3,7-Dihydroxychromone
+
COOH
OH
OH
Resorcylic acid
alkaline oxidation
HO
O
CH₂
OH
C
C
CH₂
H
HO
OH
Brazilin
fusion with KOH
mild oxidation
HCl
Methylation
HO
O
CH₂
OH
C
C
CH₂
C
C
O
HO
Brazilein
H₃CO
O
CH₂
OH
C
C
CH₂
H
H₃CO
OCH₃
Trimethyl brazilin
KMnO₄
CrO₃
Cl
HO
O
CH₂
HO
OH
Isobrazilein chloride
H₃CO
O
C=O
C=O
CH₂
H₃CO
OCH₃
KMnO₄

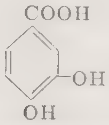


Isobrazilein chloride

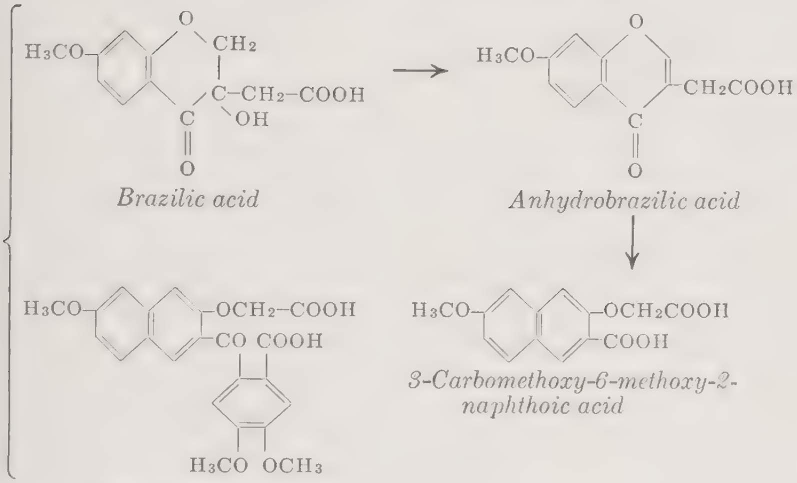


Resorcinol

+



Protocatechuic acid

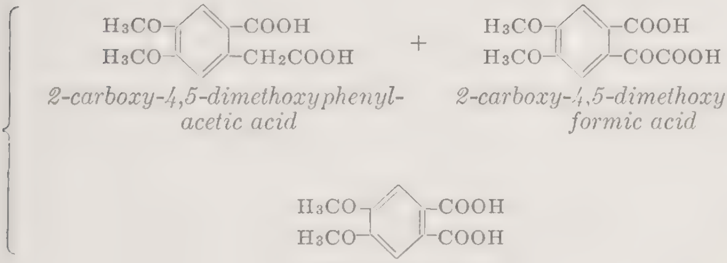


Brazilic acid

Anhydrobrazilic acid

3-Carbomethoxy-6-methoxy-2-naphthoic acid

Brazilinic acid

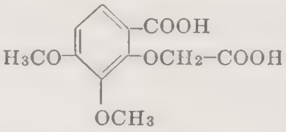


2-carboxy-4,5-dimethoxyphenylacetic acid

2-carboxy-4,5-dimethoxybenzoylformic acid

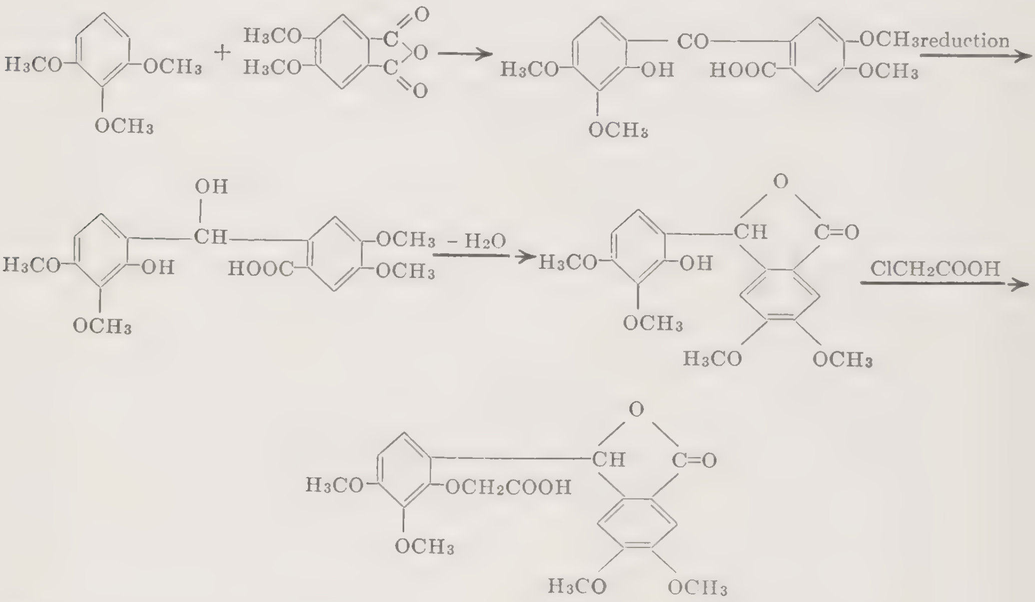
m-Hemipinic acid

hemipinic acid and a second fragment, 1-carboxy-4,5-dimethoxy-6-phenoxy-acetic acid (I) was also in agreement with this view.

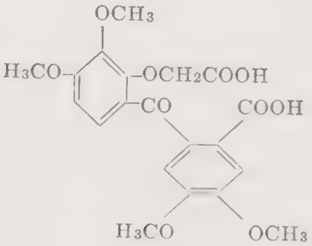


(I)

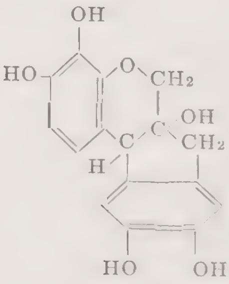
Haematoxylinic acid,[418] $C_{20}H_{20}O_{10}$, appeared to provide still another oxidative fragment in which the original skeleton had undergone less modification. This acid was reduced to a further acid,[419] $C_{20}H_{20}O_9$, which was characterized as a monobasic lactonic acid, the constitution of which was finally established by the following synthesis:



The constitution of haematoxylinic acid must be therefore (I):



(I)



(II)

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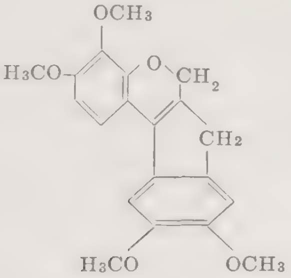
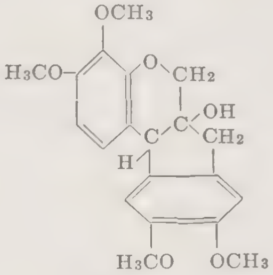
[418] Gilbody, W. H. Perkin, Jr., Yates: *Proc. Chem. Soc. London*, **16**, 105 (1900).
[419] W. H. Perkin, Jr., Yates: *J. Chem. Soc.*, **81**, 235 (1902).

Further elucidation of the constitution and discussion of the origin of fission products brought forward no further arguments beyond those already elaborated on the basis of research on brazilin, since all the products obtained differed from those of brazilin only by virtue of the additional hydroxyl group. Thus the formula (II), corresponding to that of brazilin, was finally proposed, first by Pfeiffer[421] and supported by Perkin,[420] while other workers, such as v. Kostanecki and Herzig,[422] associated with the investigation proposed constitutions corresponding to their formulas for brazilin. As with brazilin itself, the formation of a brazane derivative[423] was of some significance, but more convincing are the syntheses[424] of tetramethylanhydrohaematoxylin (III) and tetramethyldesoxyhaematoxylin (IV) which have been accomplished:



(III)

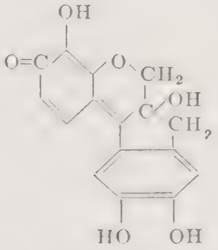


(IV)

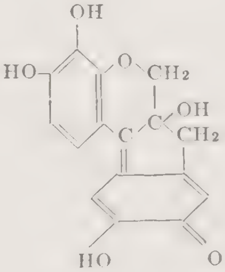
Haematein is formed by oxidation of haematoxylin according to the equation:

$$C_{16}H_{14}O_6 + O = C_{16}H_{12}O_6 + H_2O$$

and possesses by analogy with brazilein one of the structures (V) or (VI), of which (VI) is preferred (see brazalein):



(V)



(VI)

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[420] W. H. Perkin, Jr., Robinson: *Ibid.*, **93**, 489 (1908).

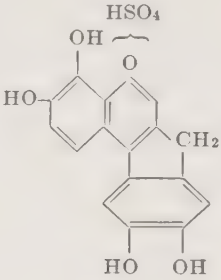
[421] Werner, Pfeiffer: *Chem. Z.*, **3**, 388, 420 (1904); W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **95**, 381 (1909); Engels, Perkin, Jr., Robinson: *Ibid.*, **93**, 1115 (1908); Pfeiffer, Haack, Willems: *Ber.*, **61**, 294 (1928).

[422] Bollina, v. Kostanecki, Tambor: *Ber.*, **35**, 1675 (1902); Herzig, Pollak: *Monatsh.*, **27**, 743 (1906); Herzig: *Monatsh.*, **16**, 906 (1895); Gilbody, W. H. Perkin, Jr., *J. Chem. Soc.*, **81**, 1040 (1902).

[423] v. Kostanecki, Rost: *Ber.*, **36**, 2202 (1903); Herzig, Pollak: *Ber.*, **37**, 631 (1904).

[424] Synthesis of *o*-diethylhaematoxylin: W. H. Perkin, Jr., Pollard, Robinson: *J. Chem. Soc.*, **1937**, 49.

As was described under brazilein, haematein is obtained by passing air through an ammoniacal solution of haematoxylin and forms red scintillating crystals. The close relationship with brazilein is constantly reflected in its reactions; thus haematein is converted, by treatment with sulfuric or hydrochloric acid, into derivatives of the isohaematein series for the salts of which constitution [425] (VII) is to be assumed. Haematoxylin is best obtained by extracting logwood with ether.



(VII)

Uses of logwood extracts: The extracts like those of brazil-wood are frequently allowed to ferment before use. Logwood dyes are still in use today in calico printing and for dyeing silk and wool, as their low price and tinctorial strength and the evenness and pleasing nature of the dyeings are important considerations. Aluminum mordants [426] give greyish violet shades, chromium dark blue to black and iron grey to black tones, grey or black dyeings being chiefly sought, usually in association with other dyestuffs. As a wool dye its application has been severely restricted by the advent of synthetic colors, but it has so far retained its position as a dyestuff for silk and is also used to dye furs and skins. The annual world consumption of logwood amounts to about 70,000 tons. The dye may also be used as an indicator.

Xanthone Pigments

If two benzene residues are fused to γ-pyrone in the α-β-positions, the skeleton of xanthone (I) is obtained from which a number of natural pigments are derived.

Euxanthone (II), $C_{13}H_8O_4$ (1,7-dihydroxyxanthone), occurs in association with glycuronic acid as the calcium or magnesium salt of euxanthic acid, also known under the trade names piuri, puree or Indian yellow.

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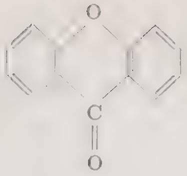
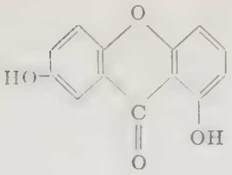
[425] Engels, W. H. Perkin, Jr., Robinson: *J. Chem. Soc.*, **93**, 1115 (1908); cf. W. H. Perkin, Jr., Robinson, Turner: *J. Chem. Soc.*, **93**, 1085 (1908).

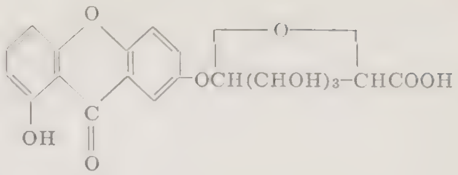
[426] Estimation of the dyestuff: Mafat: *Bull. Soc. Ind. Mulhouse*, **61**, 361 (1891); Aglot: *Angew. Chem.*, **11**, 186 (1898); v. Cochenhausen: *Angew. Chem.*, **17**, 877 (1904); Condensation product of haematoxylin with formaldehyde: D. R. P. 155,630 (Lepetit), *Friedl.*, **7**, 576.



(I)

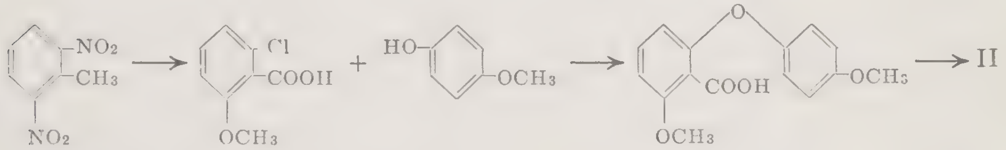


(II)

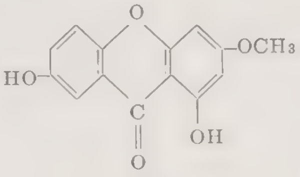


(III)

Euxanthic acid was formerly valued as a water-color pigment and was obtained by feeding [427] the leaves of *Mangifera indica* to cows; the coloring matter was excreted in the urine to the extent of 56 grams per day. It has the empirical formula [428] $C_{19}H_{16}O_{10}$ (III), and forms yellow leaflets (m.p. 162°). In accordance with constitution (III) it breaks down into glycuronic acid, $C_6H_{10}O_7$, and euxanthone [429] (yellow needles, m.p. 240°), the structure of which [430] was established by the following synthesis [431]:



Euxanthone has more recently been found in the heartwood of *Platonia insignis* Mart.[432] (geelhart or pakoeli) and is obtained by subjecting Indian yellow to hydrolysis.



(I)

Gentisin (I), $C_{14}H_{10}O_5$ (1,7-dihydroxy-3-methoxyxanthone, yellow needles, m.p. 315°), is the yellow coloring matter [433] of *Gentiana lutea*, gentian root. Examination [434] has made it probable that gentisin is the 3-methyl ether, as the synthesis of the 7-methyl ether [435] from phloroglucinol and 2-hydroxy-5-methoxybenzonitrile resulted in a compound which was not identical with gentisin, and position 1 for the methoxyl group cannot be seriously considered. Nakaoki[436] describes its occurrence in the Japanese drug To-Yaku prepared from *Swertia japonica* Makino, but quotes the melting point as 267°. Gentisin is prepared from an alcoholic extract of gentian root. It is contaminated by another xanthone pigment, the so-called datiscetin (q.v.).

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[427] Preparation: Rupe: "Chemie der natürlichen Farbstoffe," Vol. **I,** p. 11.

[428] Neuberg, Neimann: *Z. physiol. Chem.*, **44,** 115 (1905); Robertson, Waters: *J. Chem. Soc.*, 1931, 1709; cf. Wiechowski: *Arch. exp. Path. Pharmakol.*, **97,** 462 (1923); F. Mayer: *Ibid.*, **101,** 383 (1924).

[429] Literature: V. Meyer und P. Jacobson, "Lehrbuch der organischen Chemie," **II,** 3, p. 763; the pigment is used in India for painting walls and doors.

[430] Literature: Schultz: "Farbstofftabellen," 7th ed., Vol. **I,** p. 632, No. 1372.

[431] Ullmann and Panchaud: *Ann.*, **350,** 108 (1906).

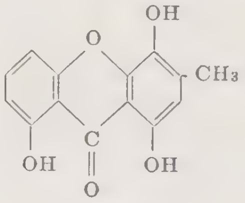
[432] Spoelstra, van Royen: *Rec. trav. chim.*, **48,** 370 (1929).

[433] Literature: V. Meyer und P. Jacobson: "Lehrbuch der organischen Chemie," Vol. **II,** 3, p. 764.

[434] A. G. Perkin: *J. Chem. Soc.*, **73,** 672, 1028 (1898).

[435] Shinoda: *J. Pharm. Soc. (Japan)*, **1926,** 89; *J. Chem. Soc.*, **1927,** 1983.

Ravenilin.[437] This compound, $C_{14}H_{10}O_5$, which forms yellow prismatic needles (m.p. 267-268°), has been isolated from the dried mycelium of *Helminthosporium Ravenelii* Curtis and *Helminthosporium turcicum Passerini.* Its constitution has been shown by degradation and synthesis to be 3-methyl-1,4,8-trihydroxyxanthone:



Ravenelin

Mangostin[438] (yellow needles, m.p. 181°) occurs in the fruit skins of *Garcinia mangostana,* a tropical tree of the *Guttiferae* family. Numerous researches into the constitution led to the empirical formulas $C_{20}H_{22}O_5$ (Liechti, also Yamashiro[439]), $C_{23}H_{24}O_6$ (van Scherpenberg and Hill, also Murakami[440]), $C_{16}H_{16}O_4$ (Dekker), and $C_{21}H_{24}O_5$ (Dragendorff), but the most satisfactory progress was made by Murakami who was able to utilize the results of the earlier workers. His formula, $C_{23}H_{24}O_6$, is supported by determinations of the molecular weight based on the introduction of foreign elements into the molecule. Mangostin contains one methoxyl group and three hydroxyl groups, of which at least one appears to be aromatic and is comparable with a hydroxyl group adjacent to a carbonyl group in hydroxyxanthones or hydroxyflavones. Hydrogenation yielded a tetrahydroderivative, and when treated with alcoholic potash, mangostin yielded isoamyl alcohol, methylheptenol and a phenolic product, $C_{12}H_{16}O_3$, containing one methoxyl group and two hydroxyl groups. The dimethyl compound of this phenol gave 2,3,5-trimethoxybenzoic acid (the constitution was proved by synthesis) on oxidation, and its dihydroderivative gave isocapronic acid by oxidation, so that its structure must be (I).

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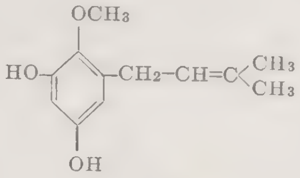
[436] Nakaoki: *J. Pharm. Soc. (Japan),* **1927,** 27.

[437] Raistrick, Robinson, White: *Biochem. J.,* **30,** 1303 (1936).

[438] Schmid: *Ann.,* **93,** 83 (1855); Liechti: *Arch. Pharm.,* **229,** 426 (1891); Scherpenberg: *Rec. trav. chim.,* **35,** 346 (1915); Hill: *J. Chem. Soc.,* **107,** 595 (1915); Dekker: *Rec. trav. chim.,* **43,** 727 (1924); Dragendorff: *Ann.,* **482,** 280 (1930); **487,** 62 (1931).

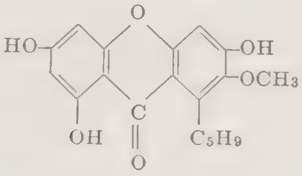
[439] Yamashiro: *Bull. Chem. Soc. (Japan),* **7,** 1 (1932).

[440] Murakami: *Proc. Imp. Acad. (Tokyo),* **7,** 254, 311 1931); *Ann.,* **496,** 122 (1932).

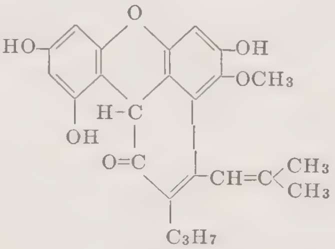


(I)

Yamashiro had been able to fuse mangostin with alkali and so to isolate a compound containing one methoxyl and three hydroxyl groups, to which he assigned the molecular formula $C_{16}H_{15}O_5$; repeated fusion with alkali yielded phloroglucinol. Murakami now gives this compound the formula $C_{19}H_{18}O_6$, constituting it as the xanthone derivative (II):



(II)



(III)

The formula of mangostin differs from (II) only in containing an additional C_4H_6 residue which would appear to be attached to the α-carbon atom of the C_5H_9 chain (cf. formation of methylheptenol from mangostin). Remembering now the production of isocapronic acid by oxidation with potassium permanganate, consideration of these further observations leads to formula (III). Mangostin is isolated by extracting the skins with alcohol, evaporating the solution and freeing the crystals which separate from resinous material.

α-Pyrone Pigments

Daphnetin,[441] $C_9H_6O_4$ (7,8-dihydroxycumarin, yellow needles, m.p. 256°), occurs as its 7-glucoside daphnin, $C_{15}H_{16}O_9$ (colorless prisms, decomposing at 228-229°), in the bark of *Daphne alpina, Daphne mezereum* (*mezereon* or laurel herb) and in *Daphne odora* Thunberg. Its constitution has been confirmed by synthesis[442] from pyrogallol and malic acid and also from pyrogallol aldehyde and sodium acetate:

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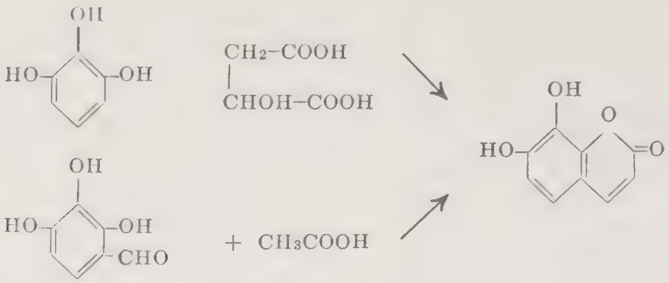
[441] Zwenger: *Ann.*, 115, 1 (1860); Stünkel: *Ber.*, 12, 109 (1879); v. Pechmann: *Ber.*, 17, 929 (1884); Wessely, Sturm: *Ber.*, 63, 1299 (1930); Asai: *Acta phytochimica*, 5, 9 (1930).

[442] v. Pechmann: *Ber.*, 17, 929 (1884); Gattermann, Köbner: *Ber.*, 32, 287 (1899).



Daphnetin dyes chromium- and aluminum-mordanted wool an olive-yellow shade. Its role in the plant appears to be one of protection against rays of short-wave length.

Nothing is known about the tinctorial properties of aesculetein and scopoletin [443] which are constitutionally allied to daphnetin.

PIGMENTS OF UNKNOWN CONSTITUTION NOT CONTAINING NITROGEN

In this section are collected those non-nitrogenous coloring matters of which the constitution is unknown. As no chemical classification is possible they are arranged, as far as the literature provides any clue, according to their origin.

Coloring Matters of Flowers

Hibiscetin [1] (yellow leaflets, m.p. 340°) occurs in the flowers of *Hibiscus Sabdariffa* (red sorrel) (West Indies) accompanied by gossypetin and quercetin. Hibiscetin is soluble in alkali to a yellow solution, forms an acetyl derivative (m.p. 238-239°) and may be identical with hibiscin (q.v.).

Hypericin or Hypericum red.[2] Flowers of *Hypericum perforatum* (St. John's wort) contain, in addition to quercetin, a red coloring principle, hypericin or hypericum red. Hypericin was obtained as a dark violet-red powder with the formula $C_{16}H_{10}O_5$, which suggested its belonging to the flavone group. A syrup, which possibly consisted of acetophenone and benzoic acid, was obtained by fission with alkali. In a recent publication Brockmann, Haschad, Maier and Pohl [3] have given a preliminary account of the properties of a crystalline preparation of hypericin obtained from *Hypericum perforatum*. The dark violet or black, thin, glistening needles decompose at about 300° without melting, and give analytical values agreeing with the formula $C_{28}H_{16}O_8$. It is difficultly soluble in the common organic solvents, except pyridine and piperidine, to give red solutions [absorption bands 597-554-516 mμ (ethyl acetate)]. Its solutions are red in phosphate buffer solution at pH 8.6 but are green in caustic alkali or in concentrated sulfuric acid. A crystalline acetyl compound (decomposing without melting) and a yellow crystalline benzoyl compound (m.p. 224-226°) were also obtained; from the analysis of the latter it was deduced that hypericin contains six hydroxyl groups, and as no methoxyl groups are present it is presumed that the remaining two atoms of oxygen belong to a quinone grouping. From consideration of its molecular formula hypericin can be no simple polyhydroxyanthraquinone, and Brockmann suggests that it is probably a hexahydroxy- derivative of meso-dianthrone, $C_{28}H_{16}O_2$. More remarkable than the chemical results, however, is the demonstration that the photodynamic action of hypericin is almost certainly responsible for the sensitiveness to light, sometimes severe and even fatal, of animals which have eaten *Hypericum* species.

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[443] Bergmann in Klein: "Handbuch der Pflanzenanalyse," Vol. **III**, 2, p. 827.

[1] A. G. Perkin: *J. Chem. Soc.*, **95**, 1855 (1909); Hadders and Wehmer suggest the formula $C_{15}H_{10}O_8$ in Klein, "Handbuch der Pflanzenanalyse," Vol. **III**, 2, p. 941.

[2] Dieterich: *Pharm. Zentralhalle*, **32**, 683 (1891); Wolff: *Ibid.*, **36**, 193 (1895); Cerny: *Z. physiol. Chem.*, **73**, 371 (1911); cf. Keegan: *Chem. News*, **111**, 290 (1915).

[3] Brockmann, Haschad, Maier, Pohl: *Naturwiss.*, **27**, 550 (1939).

Trifolitin.[4] In addition to the flavone derivative pratol, the flowers of red clover, *Trifolium pratense*, contain a glycoside trifolin, $C_{22}H_{22}O_{11}$ (yellowish needles decomposing at 260°), which on hydrolysis breaks down into rhamnose and trifolitin ($C_{16}H_{10}O_6$, yellow needles decomposing at 275°). Trifolitin dissolves in alkalies to a yellow and in sulfuric acid to a bright fluorescent green solution. It forms a tetraacetyl derivative (m.p. 182°), contains no methoxyl groups and possesses the properties of a tetrahydroxyphenyl-naphthoquinone. Pale-yellow dyeings on mordanted cotton may be obtained. Trifolitin is obtained from an alcoholic extract of the flowers.

Coloring Matters of Leaves

Coca pigments.[5] Coca leaves contain: *Cocacitrin*, $C_{28}H_{32}O_{17}$ (pale-yellow prisms, m.p. 186°), which undergoes fission into a sugar cocaose (α-talose, probably formed from a biose) and *cocacetin*, $C_{16}H_{12}O_7$ (yellow needles, m.p. 261-263°); the latter may be degraded by alkali to phloroglucinol and protocatechuic acid. Again the mother-liquors from cocacitrin contain another pigment, *cocaflavin*, $C_{34}H_{38}O_{19}$ (m.p. 163-164°), of which identified fragments are glucose and galactose, and *cocaflavetin*, $C_{20}H_{12}O_7(OCH_3)_2$, (pale-yellow leaflets, m.p. 230°). These pigments may be obtained by protracted extraction of Java coca.

Cuscutin.[6] This has been isolated from the parasitic medicinal plants *Cuscuta epythymum* and *Cuscuta reflexa* Roxb. (*Convolvulaceae*) which flourish on Indian shrubs. Cuscutin, $C_{15}H_{12}O_9$, is characterized by two phenolic groups, forming dimethyl and diacetyl derivatives, but does not appear to be a flavone derivative.

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[4] Power, Salway: *J. Chem. Soc.*, **97**, 231 (1910).

[5] Hesse: *J. prakt. Chem. (2)*, **66**, 401 (1902).

[6] Barber: *J. pharm. chim. (6)*, **2**. 107 (1895); Agarwal, Dutt: *J. Indian Chem. Soc.*, **12**, 384 (1935)

Coloring matters of Digitalis lutea.[7] A yellow pigment which is soluble in alkalies has been isolated from the residues from the preparation of digitalin. It crystallizes in yellow needles (m.p. 217-218)°, and has the composition and molecular weight agreeing with $C_{16}H_{12}O_4$. A compound, $C_{16}H_{12}O_7$, forming pale-yellow needles (m.p. 225-227°), has been found in *Gossypium indicum*.[8]

Cactorubin.[9] In the presence of moisture and oxygen, surface wounds of cacti develop a seal- or carmine-red pigment, cactorubin. No relation to carminic acid has yet been demonstrated.

Oroberol and orbol.[10] *Orobus tuberosus*, one of the *Papilionaceae*, yields a crystalline pigment termed oroberol, $C_{18}H_{14}O_8$ (pale rose-colored leaflets, m.p. 290°), which is not a glycoside but an acid containing either two carboxyl groups or one carboxyl group and a labile lactone ring. It is prepared from an alcoholic extract. Simultaneously a β-glucoside, oroboside (pale-yellow prisms, m.p. 220-221°), was obtained and the formula $C_{21}H_{20}O_{11}$ was confirmed by determinations of the molecular weight. It is broken down by hydrolysis into glucose and orobol, $C_{15}H_{10}O_6$ (yellow needles, m.p. 270.5°), which is apparently a tetrahydroxyflavone in which, to judge from its sensitiveness to oxidation two hydroxyl groups are in adjacent positions.

Osajin[11] is a yellow coloring matter (m.p. 182-186°) obtained from Osage oranges (*Maclura pomifera*) and was first thought to have the formula $C_{24}H_{22}O(COOH)(OH)_2$. Recent work indicates an isoflavone structure.

Prupersin.[12] Peach leaves (*Folia pruni persicae*) which had been dried in the shade contained a glycoside belonging to the gluco-tannoid or condensed tannin group; on hydrolysis it yielded glucose and the pigment prupersin.

Shibuol,[13] $C_{14}H_{20}O_9$, is obtained from "kakischibu." It is degraded by alkali into phloroglucinol and a compound $C_{12}H_8O_5$, which resembles the so-called kagigoma, $C_{11}H_9O_5$, obtained from the fruit, doyo-hatiya. Shibuol furnishes tetraacetyl and tetramethyl derivatives and is thus a tetrahydroxy compound. Alkali fission of tetramethylshibuol gave formic, acetic and butyric acids, the trimethyl ether of gallic acid and destetramethylshibuol (m.p. 298-299°), the last of which on fusion with potash yielded phloroglucinol, the 3-methyl ether of gallic acid, and amorphous products.

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[7] Adrian, Trillat: *Compt. rend.*, **129**, 889 (1899).

[8] Neelakantam, Seshadri: *Chem. Zentr.*, **1937**, I, 887.

[9] Molisch: *Ber. deut. bot. Ges.*, **46**, 205 (1928).

[10] Bridel, Charaux: *Compt. rend.*, **190**, 202 (1930); *Bull. Soc. Chim. biol.*, **12**, 317 (1930); *Compt. rend.*, **190**, 387 (1930); *J. pharm. chim. (8)*, **11**, 321, 369, 417 (1930).

[11] Walter, Wolfrom, Hess: *J. Am. Chem. Soc.*, **60**, 574 (1938).

[12] Inuki: *Fol. pharmacol. (Japan)*, **4**, 446 (1927); *Ber. gesamte Biol.*, **41**, 335 (1927).

[13] Komatsu, Matsunami: *Mem. Coll. Sci., Kyoto Imp. Univ. (A)*, **7**, 15 (1925); **11**, 205 (1928); Komatsu, Matsunami, Kurata: *Ibid.*, **11**, 211 (1928); Komatsu, Kurata: *Ibid.*, **13**, 323 (1931).

Xanthomicrol.[14] This pigment is obtained from *Micromeria Chamissonis* Greene, a plant growing on the Pacific coast of North America, where it is known as *Yerba buena.* Xanthomicrol, $C_{15}H_{10}O_4(OH)_2$, forms yellow needles (m.p. 225°).

Coloring Matters of Wood and Bark

Adinin,[15] $C_{16}H_{14}O_7$ (golden-yellow glistening needles, decomposing above 200°), is a product contained in the wood of *Adina cordifolia* Hok, a tree native to India (Bengal, Assam) and Burma. Experiments to isolate the coloring matter of ebony [16] appear not to have reached a successful conclusion.

Pigment of bethabarra-wood.[17] Bethabarra-wood from the west coast of Africa contains a coloring matter which has been obtained as yellow needles melting at 139.5-140.5°. The substance is identical with lapachol. The pigment is obtained by extracting the wood with water or dilute soda solution.

Dossetin,[18] is a product of the evergreen tree Doss (*Ilex Mertensii* Maxim) found on the Japanese islands Ogasawara and Okinawa. It has the composition agreeing with $C_{15}H_9O_5$ (yellow needles, m.p. 271-272°), dissolves in concentrated sulfuric acid to a dark yellow solution, and gives yellow dyeings on wool mordanted with alum, cotton or silk. Dossetin is isolated by aqueous extraction of the wood.

Excoecarin and jacarandin.[19] Both of these pigments are found in the so-called green ebony of *Excoecaria glandulosa* (family of *Euphorbiaceae*) and *Jacaranda ovalifolia* (*Bignoniaceae* family) indigenous to Jamaica and the West Indies. The wood is hard, of an orange-yellow color and stains the hands on being freshly cut. It was formerly used to a limited extent in England to dye leather and wool and to impart yellow shades to silk, but it has been supplanted by synthetic dyestuffs. Excoecarin, $C_{13}H_{12}O_5$, forms glistening lemon-yellow needles (m.p. 219-221°), which dissolve in alkalies to a violet-red solution or in ammonia to a brown solution. It dyes textiles of animal origin yellow, possesses no methoxyl groups, but forms a tribenzoyl derivative $C_{13}H_9O_5(C_6H_5CO)_3$ (colorless needles, m.p. 168-171°) and a dimethyl ether, $C_{13}H_{10}O_3$-

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[14] Power, Salway: *J. Am. Chem. Soc.*, **30**, 253 (1908)

[15] Lal, Dutt: *J. Indian Chem. Soc.*, **12**, 257 (1935).

[16] Wedekind: *Ber.*, **68**, 2363 (1935); cf. Hilpert, Wislinck: *Ber.*, **69**, 680 (1936); Tinbergen, van der Vloodt [*Chem. Weekblad*, **34**, 254 (1937)] mention a red pigment appearing in Sereh disease of sugar-cane.

[17] Sadtler, Rowland: *Am. Chem. J.*, **3**, 22 (1881/2).

[18] Ito: *J. Coll. Eng. Tokyo Imp. Univ.*, **4**, 57 (1908).

[19] A. G. Perkin, Briggs: *J. Chem. Soc.*, **81**, 210 (1902); A. G. Perkin: *J. Chem. Soc.*, **103**, 657 (1913).

$(OCH_3)_2$ (shining yellow needles, m.p. 117-119°). The molecular weight of the dimethyl ether proved to be in agreement with the formula assigned. Fusion of the pigment with potash gave 1-methyl-2,5-dihydroxybenzene and 2,5-dihydroxybenzoic acid, the second product probably arising by secondary oxidation of the first. Bromine oxidizes the pigment to excoecarone, $C_{13}H_{10}O_5$, copper-colored needles or leaflets (m.p. 250°), which is reconverted into excoecarin by sodium bisulfite. Excoecarone and excoecarin are thus related as quinone to hydroquinone, and indeed excoecarin forms with quinone a quinhydrone (m.p. 190°). Jacarandin, $C_{14}H_{12}O_5$, forms glittering platelets or needles, decomposing at 243-245° after progressive softening above 220°. It is only sparingly soluble, with a green fluorescence, in the common solvents but dissolves in sulfuric acid to orange and in alkalies to orange-red solutions, readily dyes mordanted cotton, but dyes textiles of animal origin only a weak yellow. Jacarandin possesses no methoxyl groups but gives a diacetyl derivative, $C_{14}H_{10}O_5(CH_3CO)_2$ (small yellow needles, m.p. 192-194°), sparingly soluble in alcohol, and a dibenzoyl derivative, $C_{14}H_{10}O_5[C_6H_5(CO)_2]$ (yellow prisms, m.p. 167-169°).

Commencing with an aqueous extract of 8 kg of finely divided wood, 17 grams of excoecarin and 3.15 grams of jacarandin (crude pigment) are obtained, but their isolation is arduous.

Locao (Chinese green). This pigment [20] occurs in *Rhamnus* varieties of which two in particular are found in China, *Rhamnus chlorophorus* and *Rhamnus utilis* (Hong pi lo chou and Pé pi lo chou). Besides Cloëz and Guignet,[21] who coined the termed *locain,* assigned the formula $C_{28}H_{34}O_{17}$, and recognized the compound as a glycoside, the constitution of the material has also been the subject of investigation by Kayser [22] and Rüdiger.[23] The results of the latter workers are here considered in a comparative sense as they are not in complete mutual agreement.

Kayser concluded that he was dealing with the ammonium salt of an acid and hence termed the pigment locaonic acid. The free acid obtained by the agency of oxalic acid was a blue-black powder of apparent formula $C_{42}H_{48}O_{27}$ (Rüdiger $C_{42}H_{46}O_{25}$). Diammonium, potassium, barium and lead salts were also obtained. Locaonic acid could be hydrolyzed by dilute sulfuric acid breaking down into locanic acid (the locaetin of Cloëz and Guignet), locaose ($C_{36}H_{36}O_{21}$) (Kayser, Rüdiger), and a sugar termed locaose by Kayser and recognized as rhamnose by Rüdiger. The fission was represented according to Rüdiger, therefore, by the equation:

$$C_{42}H_{46}O_{25} + H_2O = C_6H_{12}O_5 + C_{36}H_{36}O_{21}$$

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[20] Koechlin-Schuch in Mulhouse found the pigment in a Chinese cotton material

[21] Cloëz, Guignet: *Ber.*, 5, 388 (1872); *Jahresbericht v. Liebig u. Kopp*, 25, 1068 (1872).

[22] Kayser: *Ber.*, 18, 3417 (1885).

[23] Rüdiger: *Arch. Pharm.*, 252, 165 (1914) (bibliography).

This would lend greater probability to his formula for locain than to that of Kayser.

Locanic acid is a violet-black powder which is soluble in dilute alkalies with a violet-blue solution and of which a number of salts have been prepared. It contains one methoxyl group. If locanic acid is treated with hot 50-per cent potassium hydroxide, delocanic acid, $C_{15}H_9O_5$ (Kayser) or $C_{12}H_8O_5$ (Rüdiger) is produced, together with phloroglucinol. Delocanic acid also contains one methoxyl group but no free hydroxyl groups. The same compound is obtained, accompanied by nitrophloroglucinol and a compound $C_8H_7O_5N$ (orange-yellow needles, m.p. 129°), probably essentially 6-nitro-3-methoxybenzoic acid, by treating locanic acid with dilute nitric acid. Rüdiger held the opinion that locanic acid is a flavone derivative but, on the other hand, Bridel and Charaux [24] later isolated an unstable glycoside rhamnicoside, $C_{26}H_{30}O_{15}$ (colorless needles) from the stock-bark of buckthorn (*Rhamnus cathartica*) and other Rhamnus species, and regarded this as the parent substance of Chinese green. It breaks down into primoverose and rhamnicogenol, $C_{15}H_{12}O_6$, a leather-yellow powder (m.p. 177°) which seems to be pentahydroxymethylanthranol. To obtain the pigment, Cloëz and Guignet recommend extracting the bark of the branches and roots with hot water and adding potash or milk of lime to the extract. The dyestuff is then taken up from this mixture on large quantities of cotton, the amount adsorbed progressively increasing by repeating the dyeing as many as 10-20 times. The material is then washed with cold water while being pressed and rubbed; the dyestuff liberated is collected, spread on paper and dried. It then forms thin blue leaflets with a violet or green sheen and may contain as much as 50 per cent of ash. Further purification is effected by repeatedly extracting the crude pigment with ammonium carbonate, precipitating the extract with alcohol, and then dissolving the blue product in water and precipitating with alcohol several times. Finally, a solution so obtained (Rüdiger) is treated with ammonia and cautiously evaporated until crystallization commences, when glistening bronze crystals are obtained.

Locao is used by the Chinese as a dyestuff for cotton and silk, to which it imparts a beautiful bluish green [25] shade of excellent light-fastness. It dyes cotton from a weakly alkaline bath and may also be applied as a vat dyestuff to yield blue shades. The dyeings are, according to Rüdiger, fast to acid and alkali. It is stated by Bridel and Charaux that dyeings may also be carried out by treating cotton or silk with the colorless solution of the crystals isolated by the action of alkali on rhamnicoside, and then exposing the material to light.

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[24] Bridel, Charaux: *Compt. rend.*, 180, 857, 1047, 1219 (1925); *Ann. chim. (10)*, 4, 79 (1925), quote some interesting historical details; *Bull. Soc. Chim. Biol.*, 7, 822 (1925).

[25] For more detailed comments see Rupe, "Naturfarbstoffe," Vol. I, p. 281.

Phoenicein. *Phoenin*, $C_{14}H_{16}O_7$, is a compound present in purplewood, the wood of *Copifera bracteata* one of the family of *Caesalpiniaceae.* Phoenin [26] is converted by boiling with methyl alcoholic hydrochloric acid into phoenicein, a red pigment of formula $C_{14}H_{14}O_6$, which differs from phoenin only in lacking the elements of one molecule of water.

Sequoyin and isosequein.[27] The redwood of *Sequoia sempervivens* contains two pigments which are responsible for the fact that the freshly cut straw-yellow heart-wood quickly assumes a red or brown color. The first pigment, sequoyin, $C_{36}H_{38}O_{10}$ (reddish crystalline flocks, m.p. 214°), is hydrolyzed by 5 per cent sulfuric acid to sequein, $C_{20}H_{20}O_6$ (needles, m.p. 190°), and sequeinol, $C_{16}H_{18}O_4$ (needles decomposing at 242°). Sequein has a phenolic character, forms a hexaacetyl compound, and resembles brazilin and haematoxylin; sequeinol is also a phenol and yields a tetraacetyl compound. Isosequein (m.p. 188°) was obtained in only two instances. It was also seen to be a phenol constitutionally similar to sequein. Sequoyin and isosequein are obtained by extracting the redwood with cold water.

Pigments of Xanthoxylum flavum.[28] A yellow coloring matter of empirical formula $C_{14}H_{12}O_3$ (m.p. 133°) has been discovered in the wood of *Fagara flava*, a tree growing in the West Indies. It is constitutionally an ether-lactone and yields butyric acid on fusing with potash. Two yellow bark pigments are reported by another author,[29] of which one is described as Fagara yellow with the composition $C_{20}H_{20}O_9$. The second was found in *Ochna alboserrata* and was given the formula $C_{14}H_{13}O_5$ or $C_{14}H_{11}O_4$.

Lichen Pigments

Chiodectonic acid.[30] This coloring matter has been obtained as cherry-red scales, with a composition indicating the formula $C_{14}H_{18}O_5$, from an ethereal extract of *Chiodecton sanguineum* (*rubrocinctum*).

Destrictinic acid,[31] $C_{17}H_{18}O_7$ (black powder, m.p. 215°), occurs with other pigments in *Cladonia destricta*, although its isolation presents considerable difficulty.

Rhizocarpic and Rhizocarpinic acids,[32] $C_{28}H_{22}O_7$ (m.p. 177-179°),

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[26] Kleerekoper: *Chem. Zentr.*, **1901**, II, 858, 1085.

[27] Sherrard, Kurth: *J. Am. Chem. Soc.*, **55**, 1728 (1933).

[28] Auld, Pickles: *J. Chem. Soc.*, **101**, 1052 (1912).

[29] Greshoff: "Notizblatt des botanischen Gartens," No. 22, Berlin, 1900.

[30] Hesse: *J. prakt. Chem. (2)*, **70**, 449 (1904).

[31] Zopf: *Ann.*, **327**, 317 (1903); **346**, 82 (1906); Hesse: *J. prakt. Chem. (2)*, **83**, 22 (1911).

[32] Zopf: *Ann.*, **284**, 107 (1895); **346**, 82 (1906); Hesse: *J. prakt. Chem. (2)*, **58**, 465 (1898); *Ber.*, **30**, 357 (1897).

$C_{38}H_{26}O_9$ (m.p. 170°), respectively are found in varieties of *Lecideaceae* and *Calyciaceae* (*Rhizocarpon* lichens). Rhizocarpic acid is broken down by baryta into carbon dioxide, ethyl alcohol and phenylacetic acid, the formula resolving itself into $C_{24}H_{16}O_3(COOH)(COOC_2H_5)$. Hesse regarded unfavorably the opinion that the compound is a resorcinol ethyl pulvinic acid as he was unable to detect resorcinol on decomposing the acid; more probable seemed to him the assumption that rhizocarpic acid is ethyl dipulvinic acid, $C_{40}H_{30}O_9$, and that rhizocarpinic acid is a partial hydrolysis product arising according to the equation:

$$C_{40}H_{30}O_9 + H_2O = C_{38}H_{26}O_9 + C_2H_5OH$$

Rhizocarpic acid is obtained by extracting *Rhizocarpon geographicum* with chloroform.

Thiophanic acid,[33] $C_{12}H_{16}O_{12}$ (sulfur-yellow needles, m.p. 242°) is found in *Lecanora sordida.* It contains no methoxyl groups, but strong hydriodic acid converts it into thiophaminic acid, $C_{12}H_6O_9$, melting at 264°. Thiophanic acid is extracted from the lichen with ether, but its separation in a state of purity is troublesome.

Chiodictin,[34] *fufuracinic acid,*[35] *icmadophilic acid*[36] and *talebraic acid*[37] are lichen pigments which await more satisfactory characterization.

Coloring Matters of Resins, Drugs and Roots

Ardisiol,[38] $C_{35}H_{46}O_{10}$, from the latex of ardisin resin (Getha Adjak), a product of *Ardisia fuliginosa* Bl. (Java), consists of two orange-colored pigments, an α-form (m.p. 107°) and a β-form (m.p. 183°). The original workers suggest that these compounds are to be included in the anthraquinone series.

Coloring matter of "dragon's blood." The isolation of a pigment, $C_{17}H_{18}O_4$ (m.p. 145°), is mentioned in early investigations of the resin of the dragon's blood tree,[39] *Dracaena Draco.* More recently Hesse[40] has separated a blood-red resin from the fruit-bearing parts of the palm *Daemonorops Draco Blume,* and has isolated two pigments from this by chromatographic analysis. The main pigment, *dracocarmin,* $C_{31}H_{26}O_5$ (dark-red needles, m.p. 293°), seemed to represent a new type of anthocyanidin, and the second, *dracorubin,* $C_{28}H_{24}O_7$, forms pale-red rhombohedra decomposing at 270-280°. Both form salts with hydrochloric acid.

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[33] Hesse: *Ber.*, **30,** 357 (1897); *J. prakt. Chem. (2),* **58,** 465 (1898).

[34] Hesse: *J. prakt. Chem. (2)* **70,** 499 (1904).

[35] Zopf: *Ann.*, **338,** 35 (1905).

[36] Thies in Klein: "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 449.

[37] Hesse: *J. prakt. Chem. (2),* **68,** 1 (1903); Zopf: *Ann.*, **340,** 276 (1905); Hesse: *J. prakt. Chem. (2),* **73,** 113 (1906).

[38] Greshoff, Sack: *Pharm. Weekblad*, **40,** 127 (1903).

[39] Fraenkel, David: *Biochem. Z.*, **187,** 146 (1927); literature on other varieties of dragon's blood: Loyander: "Beiträge zur Kenntnis des Drachenblutes," Marburgh, 1887; Glenard, Boudault: *Ann.*, **48,** 343 (1843); Blyth, Hofmann: *Ann.*, **53,** 326 (1845); Boetsch: *Monatsh.*, **1,** 669 (1880); Glimmann, Tschirch: *Arch. Pharm.*, **234,** 587 (1896).

[40] Hesse: *Ann.*, **524,** 14 (1936).

The investigations of Brockmann,[41] however, present a somewhat different picture. Examining Indian dragon's blood, *Sanguis draconis,* he also obtained a dracorubin (brick-yellow needles, m.p. 315°), to which he at first assigned the formula $C_{19}H_{14}O_3$. Chromatographic analysis then indicated the presence of several pigments, and Brockmann modified his formula for dracorubin to $C_{32}H_{24}O_5$, expressing a belief that it is identical with Hesse's dracocarmine. Brockmann's dracorubin is optically active and yields acetophenone and benzoic acid on fusion with alkali. One oxygen atom is responsible for the formation of oxonium salts and another is probably to be accounted as a carbonyl group.

Flemingin.[42] This pigment is present in waras, wars or wurrus, a red resinous powder obtained from the seed cases of *Flemingia congesta,* a shrub growing in the warmer parts of India and in Africa in the neighborhood of Harrar. From there it is imported into Arabia, particularly the Yemen and Haddramant regions, where it finds application as a pigment, as a cosmetic, and also as a remedy for colds and chills. It is used like kamala, to which it bears some resemblance, to dye silk and to a smaller extent wool. The dyeing is carried out in boiling sodium carbonate solution from which golden yellow shades result. Flemingin forms orange-red needles (m.p. 171-172°) with the probable formula $C_{12}H_{12}O_3$. It dissolves in cold alkali with a deep orange-red coloration, and fusion with alkali yields salicylic acid, acetic acid and an acid of m.p. 184° (*o*-hydroxycinnamic acid ?).

Flemingin is obtained from waras, after a preliminary extraction with carbon disulfide, by extracting the residue with chloroform. The chloroform solution deposits a red precipitate and the filtrate is then evaporated to leave crude flemingin, which is crystallized from toluene. The precipitate from the chloroform solution is then treated repeatedly with chloroform, and the new extract on standing in the cold deposits a resinous material (m.p. 162-167°), of the same composition as flemingin and yielding the same degradation products on fusion with alkali. The filtrate contains a small proportion of homoflemingin (m.p. 165-166°), again of the same composition as flemingin and behaving similarly on fusion with alkali. It would appear doubtful, therefore, whether these products are not merely impure flemingin.

Gamboge.[43] This pigment is found in the latex of *Garcinia morella* (East Indies, Ceylon, Siam, Cochin China), one of the family of *Guttif-*

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[41] Brockmann, Haase: *Ber.,* 69, 1950 (1936); **70,** 1733 (1937).
[42] A. G. Perkin: *J. Chem. Soc.,* 73, 660 (1898).
[43] Schultz: "Farbstofftabellen," 7th ed., Vol. **I,**, No. 1393, p. 647; Ullmann: "Encyclopaedie der technischen Chemie," 2nd ed., Vol. **2,** p. 98.

erae (*Clusioideae*). It forms thick masses of a red-yellow color containing α-, β- and γ-garcinolic acids ($C_{23}H_{28}O_6$, $C_{25}H_{32}O_6$, and $C_{23}H_{28}O_5$) of which the γ-acid is colored red by alkali. It is used as a water-color and to color lacquers. Gamboge is gathered by making spiral incisions in the lower parts of the stems and collecting the effluent sap in bamboo tubes. These are allowed to dry for a month and heated over a fire until the contents have hardened.

Kino.[44] This is the coagulated sap of *Pterocarpus marsupium,* one of the *Papillionaceae* from the Malabar coast. It forms red-brown masses, gives a triacetate, $C_{15}H_{11}O_4\,(OCOCH_3)_3$ and a trimethyl ether, $C_{15}H_{11}O_4(OCH_3)_3$, and yields pyrocatechol and protocatechuic acid on fusion with alkali. Kino is used like catechu in dyeing and printing. Kino is obtained by making incisions in the bark of the tree and allowing the sap to harden in the air.

Nepodin,[45] $C_{18}H_{16}O_4$ (golden-yellow leaflets and needles, m.p. 158°), is found in *Rumex Nepalensis* and would seem to be a protocatechuic derivative.

Trypterin,[46] $C_{25}H_{37}O_3$ (blood-red cubes, m.p. 195°), is the coloring principle of the Chinese drug Li-Kung-Teng (*Trypterygium Wilfordii* Hook).

Coloring Matters of Fungi, etc.

Ergochrysin and ergoflavin. In extracting the alkaloids of ergot, a yellow-brown residue is obtained from which a number of yellow pigments have been isolated. Thus there is described *scelero-crystallin*[47] of formula $C_7H_7O_3$ or $C_{10}H_{10}O_4$, which is converted into a hydrate, *scelero-xanthin.* Secondly there is the so-called *ergochrysin*[48] (yellow needles) with the formula $C_{21}H_{22}O_9$, which may also yield a hydrate, $C_{21}H_{24}O_{10}$. Finally there must be mentioned *secalonic acid,*[49] a yellow pigment with formula $C_{14}H_{14}O_6$ (m.p. 248°), which likewise gives a hydrate. These compounds would seem to be identical unlike a further pigment,[50] $C_{15}H_{14}O_7$ (m.p. 350°), which is probably closely related to the flavones. Ergochrysin, the first pigment to be obtained abundantly, was isolated anew[51] from an ergot residue which unfortunately was not more exactly described. It forms golden-yellow needles (m.p. 266°) and has a formula $C_{28}H_{28}O_{12}$, which has been confirmed by determining the molecular weight. Ergochrysin is sensitive toward alkalies which convert it into amorphous products. It forms a decaacetate; dry distillation yields a phenol, $C_9H_{10}O_3$ (monoacetate, m.p. 65°), fusion with alkali gives resorcinol, 1,3,5-cresotinic acid, and 2,4,2′,4′-tetrahydroxydiphenyl, and oxidation by nitric acid gives a nitro derivative, $C_{16}H_{15}O_9N$.

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[44] Etti: *Ber.,* **11,** 1879 (1878); Simonsen: *J. Chem. Soc.,* **99,** 1530 (1911).
[45] Hesse: *Ann.,* **291,** 305 (1896); **309,** 52 (1899); Keegan: *Chem. News,* **114,** 74 (1916).
[46] Chou. Mei: *Chinese J. Physiol.,* **10,** 529 (1936).
[47] Dragendorff, Podwissotzki: *Arch. exp. Path. Pharmakol.,* **6,** 174 (1877).
[48] Jacobi: *Ibid.,* **39,** 104 (1897).
[49] Kraft: *Arch. Pharm.,* **244,** 336 (1906).
[50] Freeborn: *Pharm. J.,* *(4),* **34,** 568 (1912).
[51] E. Bergmann: *Ber.,* **65,** 1489 (1932).

The second pigment, ergoflavin,[52] is identical with the product of melting point 350° and was obtained from another ergot residue. The formula given earlier was substantiated ($C_{15}H_{14}O_7$), but instead of a tetraacetate (Freeborn) a pentaacetate (m.p. 244°) was obtained. Five out of the seven oxygen atoms are thus characterized, a result confirmed by the Zerewitinoff determination. Ergoflavin resembles vitexin. Treating with boiling potassium hydroxide leads to an acid, ergoflavinic acid, $C_{15}H_{16}O_8$ (m.p. $> 340°$) which is reconverted into ergoflavin by loss of water (lactone ?). Two red coloring matters, *sceleroerythrin* and *sceleroiodin*[53] are also known.

Gemmatein.[54] This pigment occurs as a glycoside in the fungus *Lycoperdon gemmatum* Batsch. It forms black-brown needles, $C_{17}H_{12}O_7$, which contains glucose as the sugar component. Fusion with alkali yields *p*-hydroxybenzoic acid, and homogentisinic anhydride is obtained by oxidation with hydrogen peroxide. Gemmatein is prepared from an ethereal extract of the dried fungal powder.

Monascin,[55] **monascorubin and monascoflavin**[56] (α- and β-hydroxyzerubin). The red rice, which is prepared in eastern Asia by cultivating a fungus "agkhak," one of the *Telebolae* group, or *Monascus purpureus* Wentii on rice, contains a red coloring matter[57] used in China to color beverages and foodstuffs. According to the investigation of Karrer[55] two pigments are present (as had been postulated by Hibino), one a deep red-brown material which has not yet been obtained in a state of purity, and a deep yellow compound, monascin, crystallizing in leaflets (m.p. 135-140°). Analyses and molecular weight determinations agree best with the formula $C_{24}H_{30}O_6$, but it must be remembered that monascin is very sensitive and in alkaline solution, for example, is quickly decomposed. It is free from methoxyl groups and attempts to acetylate it have not been successful. Lower fatty acids seem to occur among the oxidation products, and indeed the pigment itself would appear to possess a largely aliphatic structure. Nishikawa has isolated a so-called monascorubin, $C_{22}H_{24}O_5$ (red prisms or needles, m.p. 136°), and thence a dihydroderivative, from the fungal mycelium; both compounds are levo-rotatory. A yellow compound, monascoflavin, $C_{17}H_{22}O_4$ (rhombic leaflets, m.p. 145°), was obtained by the action of hydrogen peroxide and may also be found in old mycelia. Monascoflavin also forms a dihydroderivative and both compounds are strongly dextro-rotatory. Monascorubin contains one double linkage, a straight chain of six carbon atoms and probably a benzene nucleus; on fusion with potash it yields capronic among other aliphatic acids. These compounds are isolated, following Karrer, by extraction with ether.

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[52] E. Bergmann: *Ber.*, **65**, 1487 (1932).

[53] Literature: Zellner: "Chemie der höheren Pilze," Leipzig, 1907, cf. Tschirch: *Schweiz. Apoth. Z.*, **60**, 1 (1922).

[54] Kotake, Naito: *Z. physiol. Chem.*, **90**, 254 (1914).

[55] Salomon, Karrer: *Helv. Chim. Acta*, **15**, 18 (1932).

[56] Nishikawa: *Bull. agri. chem. soc. (Japan)*, **8**, 78 (1932).

[57] Earlier literature: Geerligs: *Chemiker Ztg.*, **19**, 1311 (1895); Went: *Ann. Sci. Nat. Botan. (8)*, **1**, 1 (1895); Wehmer: *Zentr. Bact. Parasitenk.* **(II)**, **3**, 105 (1897); Boorsma: *Chem. Zentr.*, **1896**, I, 1130; Hibino: *Proc. Koninkl. Akad. Wetenschappen, Amsterdam*, **28**, 182 1925; earlier literature is also given.

Pigment of Polysaccum pisocarpium.[58] This fungal pigment has been assigned the formula $C_{14}H_8O_6$ or $C_{10}H_6O_2$, and tetrahydroxyanthraquinone and dihydroxynaphthoquinone structures have been suggested, although in Kögl's [59] opinion the former is improbable. The pigment has, however, been obtained only in an impure condition containing ash. Another product from *Polysaccum crassipes* [60] may prove to be identical with the pigment mentioned above. Both compounds are isolated from alcoholic extracts. Thorpe [61] in 1885 mentioned a coloring matter from *Bacterium brunneum* to which the formula $C_{18}H_{14}O_3$ was assigned.

Pigments of Lactarius deliciosus. The red-brown sap of the fungus contains five or six pigments [62] (two orange-yellow, one or two green, one blue and one violet-red). They are precipitated from the fungal material by alcohol and separated by chromatographic adsorptions analysis. The violet-red material predominates. It has a composition agreeing with $C_{15}H_{14}O$ (m.p. 53°) and possesses no acid properties. Its absorption spectrum is 506-394-360 $m\mu$. Of the others it is known that the blue compound is a hydrocarbon with the molecular formula $C_{15}H_{18}$ (azulene). Finally it may be remarked that the fungus contains also the nitrogenous pigment lactoflavin.[63]

Three colored compounds have been obtained from *Lactarius deliciosus*. One, *verdazulene,* $C_{15}H_{16}$, m.p. 90°, a green crystalline hydrocarbon, is accompanied by *lactarazulene,* $C_{15}H_{18}$, a blue liquid. The third, *lactaroviolin,* $C_{15}H_{14}O$, is probably an aldehyde; to judge from the variation in the spectrum following reactions involving the carbonyl group this must be conjugated with the main chromophoric system.[63a]

Rhizopogonic acid.[64] This is a pigment isolated from the wild truffle, *Rhizopogon rubrescenz,* from the south of France. The compound, which

[58] Fritsch: *Arch. Pharm.*, **227,** 193 1889.

[59] Kögl in Klein, "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 1429.

[60] Zellner: *Sitzber. Akad. Wiss. Wien, Math.-Naturw. Kl., (IIb),* **127,** 411 (1918).

[61] Thorpe: *Chem. News,* **72,** 82 (1895).

[62] Willstaedt: *Ber.*, **68,** 333 (1935); **69,** 997 (1936).

[63] cf. Seifriz, Zetzmann: *Protoplasma,* **23,** 175 (1935) on the occurrence of a lyochrome in *Physarum polycephalum.*

[63a] Willstaedt: *Atti X° congr. intern. chim.*, **3,** 390 (1939).

[64] Oudermann: *Rec. trav. chim.*, **2,** 155 (1884).

has the formula $(C_{14}H_{18}O_2)_x$ or $(C_{20}H_{26}O_3)_x$ (red needles, m.p. 127°), is acidic in nature, dissolving in alkali with a violet-red color and forming a crystalline potassium salt with the probable formula $C_{28}H_{35}O_4K$. The acid is isolated by macerating the fungus in alcohol and extracting the residue with ether.

Fulvic acid[65] is contained in *Penicillium griseo-fulvum* Dierckx, *Penicillium flexuosum* Dale, or *Penicillium Brefeldianum* Dodge when grown on a synthetic medium. Possessing the formula $C_{14}H_{12}O_8$ and decomposing at 246°, fulvic acid forms trialkyl derivatives and may be titrated as a dibasic acid.

Geodin,[66] $C_{17}H_{12}O_7Cl_2$ (fine yellow needles, m.p. 235°), and *erdin* $C_{16}H_{16}O_7Cl_2$ (fine yellow needles, m.p. 211°), are dibasic acids found in *Aspergillus terreus.*

Rubrofusarin,[67] $C_{15}H_{12}O_5$ (orange-red needles, m.p. 210-211°), a monomethyl ether containing also one methyl group and two active hydrogen atoms, has been isolated from *Fusarium Culmorum.* The suggestion has been made that rubrofusarin is the monomethyl ether of a methyltrihydroxyxanthone. Another pigment which has been isolated, aurofusarin, $C_{30}H_{20}O_{12}$ (orange-yellow prisms which do not melt below 360°), seems to be a dimethyl ether. Other fungal and bacterial pigments,[68] for which no chemical formulas have as yet been advanced, have not been considered here.

Coloring matter of Melanargia galatea.[69] The wings of the "Marbled White" *Melanargia galatea* contain two pigments. The one has been identified as a lipochrome and shows the absorption spectrum of carotene, the other one melts at 253°, and dissolves readily in alkali or in concentrated mineral acids with a bright yellow color. When boiled with magnesium powder and hydrochloric acid it yields a pink filtrate which becomes bright yellowish green on making alkaline. The pigment, which may be a flavone or flavonole (the properties of quercetin agree fairly well with those of the pigment) was also found in cock's foot grass (*Dactylis glomerata*) one of the known food plants of the larva.

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[65] Oxford. Raistrick, Simonart: *Biochem. J.*, **29**, 1102 (1935).
[66] Raistrick, Smith: *Ibid.*, **30**, 1315 (1936).
[67] Ashley, Hobbs, Raistrick: *Ibid.*, **31**, 385 (1937).
[68] Compilative review: Kögl in Klein, "Handbuch der Pflanzenanalyse," Vol. **III**, 2, p. 1140.
[69] Thomson: *Biochem. J.*, **20**, 73, 1026 (1926).

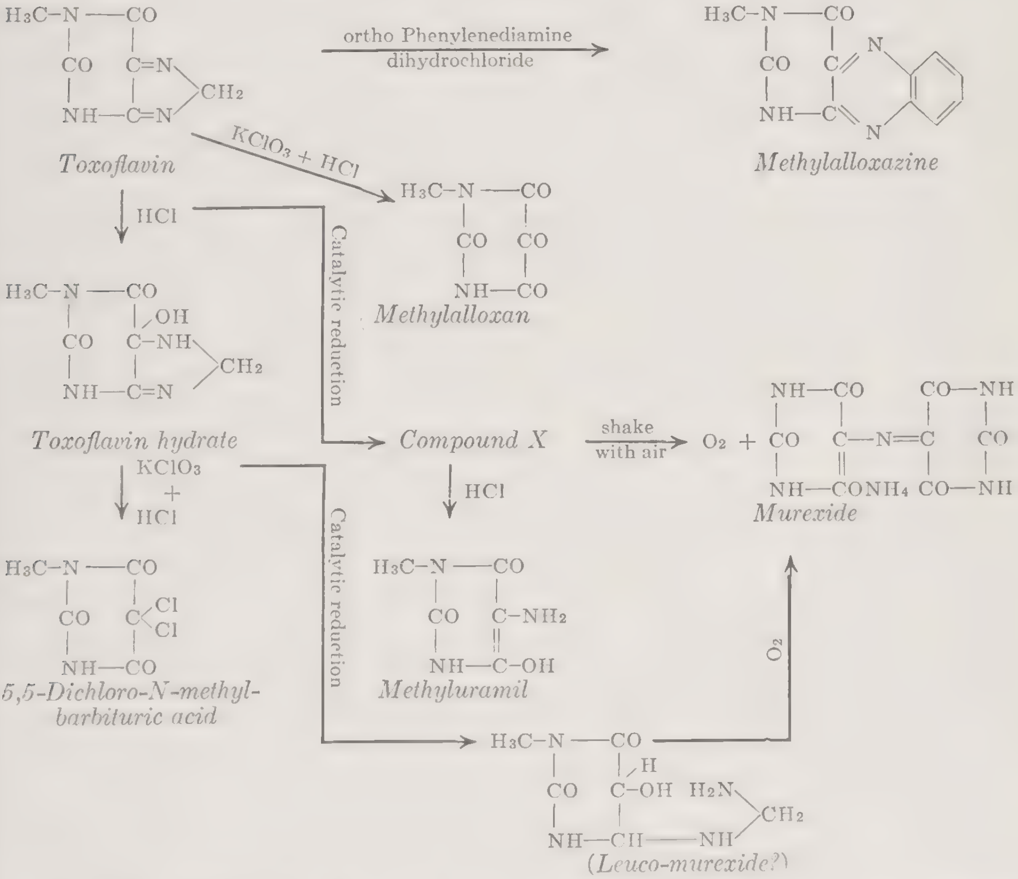
Chapter 5

Compounds Containing Heterocyclic Nitrogen Atoms

These pigments are classified in the groups: derivatives of pyrimidine, of pyrrole, of indole, of pyridine and of pyrazine. A number of pigments of unknown constitution are also included. In this section are discussed the important pigments of the blood, bile, the green leaf and also the lyochromes (flavins).

Derivatives of Pyrimidine

Toxoflavin[1] is one of two toxic principles isolated from "Bongkrek" bacteria. Bongkrek is a Central Java foodstuff prepared from coconuts,


Toxoflavin
ortho Phenylenediamine dihydrochloride
Methylalloxazine
KClO₃ + HCl
Methylalloxan
HCl
Catalytic reduction
Toxoflavin hydrate
Compound X
shake with air
O₂ +
Murexide
KClO₃ + HCl
HCl
5,5-Dichloro-N-methyl-barbituric acid
Catalytic reduction
Methyluramil
O₂
(Leuco-murexide?)

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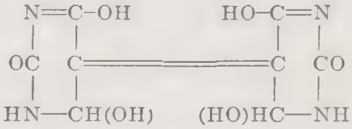
[1] van Veen, Mertens: *Rec. trav. chim.*, 53, 257, 398 (1934); van Veen, Baars: *Proc. Koninkl. Akad. Wetenschappen Amsterdam*, 40, 498 (1937); *Rec. trav. chim.*, 57, 248 (1938); cf. also Stern: *Biochem. J.*, 29, 500 (1935).

and the cause of its occasional poisonous nature was unknown until these products of bacterial metabolism were discovered. Toxoflavin, $C_6H_6O_2N_4$ (m.p. 171°), is a pale-yellow compound soluble in water with a very weak yellow-green fluorescence. It undergoes the changes shown in the preceding table and has probably the constitution given on page 263.

Certain sulfur bacteria[2] from the group of *Sulfomonas* (*Thiobacillus*) *thiooxidans* produce a red coloring matter when grown in the dark and in presence of thymine glycol. The compound is destroyed by light and, from theoretical considerations, is provisionally given the structure:



Pterins[3] (*lepidopterins*). This name was proposed by Wieland and Schöpf to describe the pigments of butterfly-wings. The determination of their constitution has been hindered by the great difficulty of gathering material and by the small amount contributed by each insect, but their inclusion in the pyrimidine group is now no longer disputable. The pterins[4] occur as small colored or colorless granules in association with chitin, the following individuals having been recognized:

In *Pieris brassicae* and *Pieris napi* (cabbage butterflies): Leucopterin.

In *Gonepteryx rhamni:* Xanthopterin, accompanied by erythropterin, leucopterin, guanopterin and chrysopterin.

In *Catopsilia rurina:* Xanthopterin and chrysopterin.

In *Appias nero* F. (Java), *Catopsilia argante* F. (S. America), *Colias edusa* F. and *Euchloe cardamines* L: Xanthopterin, erythropterin, guanopterin; the last mentioned contains also a pigment resembling leucopterin.

The pigments are purified by first removing fats and protein by treating with ether and alcohol and then dissolved in ammonia. As the only method of separating mixtures of pterins consisted in fractional extraction with ammonia or fractional precipitation with acid (chromatographic analysis was only recently introduced), and as the pterins have no characteristic melting points, the preparation of pure products was always attended by uncertainty. Furthermore the carbon and hydrogen content is practically invariable and only the nitrogen content provides some analytical characterization. A pterin is to be regarded as homogeneous only when the same product with the same properties and composition is isolated from quite differently constituted pigment mixtures from different insects, and so far this criterion is satisfied only by xanthopterin.

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[2] Baudisch: *Svensk. Kem. Tid.*, 47, 191 (1935).

[3] Wieland, Schöpf: *Ber.*, 58, 2178 (1925); older literature in Abderhalden: "Biochemisches Handlexikon," Vol. 11, p. 355; especially Hopkins: *Chem. News*, 60, 57 (1889); *Nature*, 40, 335 (1889); 45, 197, 581 (1891/2) and *Proc. Roy. Soc. London*, 57, 5 (1894/5), where the pigments of butterfly wings are first recognized as purine derivatives; *Philos. Trans. Roy. Soc. London*, 186, 661 (1895).

[4] Wieland, Schöpf: *Ber.*, 58, 2178 (1925); Schöpf, Becker: *Ann.*, 507, 266 (1933); cf. Wieland, Metzger, Schöpf, Bülow: *Ann.*, 507, 226 (1933); Schöpf, Becker: *Ann.*, 524, 49, 124 (1936).

Leucopterin.[5] This pigment is obtained from the wings of *Pieris brassicae* and *Pieris napi* (cabbage butterflies) and was at first mistaken for uric acid. Leucopterin forms colorless crystals to which the formula $C_{19}H_{19}O_{11}N_{15}$ was given; it gives the murexide reaction, and was regarded as a tetrabasic acid. The two characteristic oxidation products of uric acid, alloxan and allantoin, were not encountered, however. Treatment with phosphorous chloride afforded a reaction product, apparently $C_{19}H_{16}O_8N_{15}Cl_3$, in which three hydroxyl groups seemed to have been replaced by chlorine, and the compound also formed a triacetyl derivative with loss of one molecule of water. As uric acid decomposes on heating with concentrated hydrochloric acid at 170° in the following manner:

$$C_5H_4O_3N_4 + 5H_2O = H_2N - CH_2 - COOH + 3NH_3 + 3CO_2$$

only an incompletely balanced equation could be adduced for leucopterin

$$C_{19}H_{19}O_{11}N_{15} + 16H_2O = 3H_2N - CH_2 - COOH + 12NH_3 + 9CO_2 + 3CO + (C)$$

i.e., only 18 carbon atoms are accounted for. The result indicated, however, that three purine rings are present in the molecule. Introduction of chlorine into a methyl alcoholic suspension of leucopterin gave, by analogy with uric acid, the trihydrochloride of a hexamethyl ether, $C_{19}H_{19}O_{11}N_{15}(OCH_3)_6$, which was basic in character although the parent compound lacked basic properties. The action of chlorine water on leucopterin gave a compound, $C_{19}H_{19}O_{11}N_{15}(OH)_6$, corresponding to the glycol of uric acid, whereas chlorine in acetic acid oxidized the pigment to a diglycol, $C_{19}H_{19}O_{11}N_{15}$, in which only two pyrimidine rings entered into reaction. On treatment with baryta water this glycol underwent fission, with the production of guanidine and 3 molecules of oxalic acid. It is clear, therefore, that the relatively large amount of ammonia obtained in the decomposition with hydrochloric acid originates in the form of imino-groups, while the oxalic acid provides the carbon monoxide. It may be further deduced that carbon atoms of the purine skeleton participate in the production of oxalic acid. In the above-mentioned hexamethyl ether, 3 methoxyl groups were further replaced by hydroxyl groups, giving rise to the unstable compound $C_{19}H_{19}O_{11}N_{15}(OH)_3$-$(OCH_3)_3$, which on decomposition lost 3 molecules of carbon dioxide and one molecule of guanidine, while the reaction solution provided an ester,

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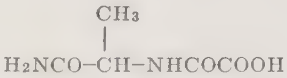
[5] Schöpf, Wieland: *Ber.*, 59, 2067 (1926); Wieland, Metzger, Schöpf, Bülow: *Ann.*, 507, 226 (1933); Schöpf, Becker: *Ann.*, 507, 266 (1933); hydrolysis with acids: Schöpf, Becker, Reichert: *Ann.*, 539, 156 (1939).

$C_4H_4O_3N_2(OCH_3)_2$, derived from an acid, $C_4H_5O_4N_2(OCH_3)$, of the structure:



$$H_2NCO\text{–}\overset{\displaystyle CH_3}{\overset{|}{C}H}\text{–}NHCOCOOH$$

i.e., an oxaminic acid in which is substituted a methoxyacetamide residue.

In spite of the experimental evidence given above, further work on the degradation products of the triglycol[6] and examination of the light adsorption[7] did not tend to place the formerly suggested formulas containing 3 pyrimidine rings on a firmer basis. Of greater importance in the determination of constitution may be experiments which demonstrate a connection between leucopterin and xanthopterin (see xanthopterin, and the structure of leucopterin there deduced).

Leucopterin is obtained by extracting the wings of *Pieris napi* with ammonia, after first extracting with ether, and then evaporating the ammoniacal solution in vacuum, whereupon the ammonium salt separates. 39.1 grams of crude leucopterin were obtained from 1164 grams of the wings, or from 215,000 insects, this corresponding to a yield of 0.19 mg from each insect.

Pigments have also been found in wasps, *Vespa crabro* (hornets), *Vespa germanica* and *Vespa vulgaris*, which exhibit characteristic properties recalling those of leucopterin (*e.g.*, composition and the murexide reaction). The yellow color of the lower parts of the body of the wasp is due to such pigments.

Xanthopterin. The analytical figures of this pigment, after liberation from the so-called β-barium salt, indicated the molecular formula $C_{19}H_{20}O_7N_{16}$, although the composition of native xanthopterin agreed with a formula containing one less molecule of water, $C_{19}H_{18}O_6N_{16}$. It gives the murexide reaction, contains no N-methyl groups, couples with diazonium salts as does xanthine, and on boiling with alkali slowly liberates ammonia. Xanthopterin exhibits absorption bands at 391-360-267-243 mμ, and although a connection with vitamin B_2 was at once suspected, it has no vitamin action on rats.[8]

Degradation with sodium chlorate[9] at 100° gave oxalic and glyoxylic acids, guanidine and urea, and if the temperature was maintained at 80°, oxalylguanidine, $HOOC—CO—NH—C(=NH)—NH_2$, could be isolated. It was concluded that at least two aminopyrimidine residues were present. Degradation with nitrous acid led to the same results and a number of other deductions were drawn from the results of acid hydrolysis leading to the formation of glycocoll.

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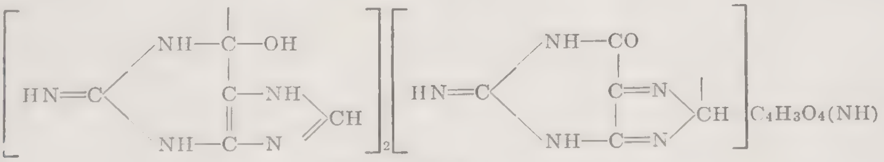
[6] Wieland, Kotzschmar: *Ann.*, 530, 152 (1937).
[7] Fromherz, Kotzschmar: *Ann.*, 534, 283 (1938).
[8] Kuhn, György, Wagner-Jauregg: *Ber.*, 66, 317 (1933) and p. 319.
[9] Schöpf, Kottler: *Ann.*, 539, 128 (1939); Schöpf, Becker, Reichert: *Ann.*, 539, 156 (1939).

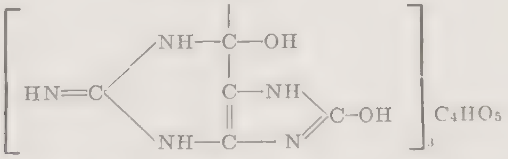
More informative, however, would appear to be the study of the action of hydrogen peroxide [10] on the pigment. The first product was a peroxide, $C_{19}H_{18}O_6N_{16} \cdot 3H_2O$, which was then converted into a compound, $C_{19}H_{20}O_{10}N_{16}$, which was no longer basic. Now leucopterin was thought to have the formula $C_{19}H_{19}O_{11}N_{15}$, and the new compound was, formally at least, an iminoleucopterin or leucopterin, in which one oxygen atom has been replaced by NH. Iminoleucopterin is now known to be leucopterin itself.[9a] When leucopterin was treated with nitrite and concentrated sulfuric acid, three C:NH groups were converted into C=O groups with the formation of desiminoleucopterin, $C_{19}H_{14}O_{13}N_{12}$; four C:NH groups in iminoleucopterin (from xanthopterin) were likewise replaceable with the production of the same desiminoleucopterin. It was said, therefore, that both compounds contain the same skeleton but that xanthopterin contains an unlocated NH= or —NH_2 in place of O or OH respectively.

If xanthopterin itself is submitted to hydrolytic fission so that this NH= or NH_2— group is removed, with the formation of a compound corresponding to leucopterin with 15 nitrogen atoms, the product has the formula $C_{19}H_{19}O_8N_{15}$, and the addition of three atoms of oxygen by oxidation with hydrogen peroxide would be necessary to convert it from the xanthopterin to the leucopterin, $C_{19}H_{19}O_{11}N_{15}$, stage. The relationship of xanthopterin to leucopterin would thus be that of xanthin to uric acid, assuming the presence of three purine nuclei. The action of hydrogen peroxide at a raised temperature leads to iminocyanuric acid, just as cyanuric acid is obtained from uric acid itself.

On the basis of the foregoing facts and further examination of leucopterin, the following partial formulas were proposed, although they were difficult to reconcile with the hexabasic acid character of xanthopterin:



Xanthopterin

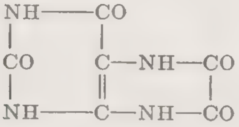


Leucopterin

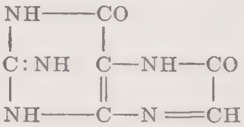
[9a] Schöpf: *Naturwiss.*, 28, 478 (1940).

[10] Wieland, Purrmann: *Ann.*, 539, 179 (1939).

The peculiar difficulties of this work, particularly the analytical difficulties, seem to have led to the acceptance of the foregoing statements when they were not in reality statements of fact. Now it is known that the compound formerly termed iminoleucopterin is actually leucopterin, that anhydroleucopterin is really desoxyleucopterin, and that isoleucopterin and pseudoleucopterin among others are only impure compounds to which no significance need be attached. The major result of this revision has been the introduction of C_6 formulas; and although it is as yet uncertain which of the foregoing statements can be rationally interpreted, two achievements definitely justify this introduction. It is clear that the degradation products which were formerly regarded as complex are merely pyrimidine derivatives. Now Purrmann has shown that 2:6-dihydroxy-4:5-diaminopyrimidine and oxalic acid afford desiminoleucopterin itself, which must therefore have constitution (A). 2:5:5-Triamino-6-hydroxypyrimidine on the other hand was first converted into a dichloroacetyl compound which on cyclization yielded xanthopterin (B) [10a]:



(A)



(B)

Xanthopterin was obtained by extracting the air-dried wings of 500 male specimens of *Gonepteryx rhamni* weighing 7.4 grams first with ether, which removed a trace of another yellow pigment of unknown constitution, and then with ammonia. The product was obtained by evaporation, the yield of crude material corresponding to $\frac{2}{3}$ mg from each insect. Xanthopterin may also be obtained with leucopterin and a third pterin from the integuments of the lower parts of the wasp (*Vespa crabro, germanica* and *vulgaris*).

Erythropterin,[11] $C_{19}H_{17\text{-}18}O_{8\text{-}10}N_{13\text{-}11}$, was obtained by the general method described above and purified by crystallization from 0.1*N* hydrochloric acid. It is remarkably soluble in water, possesses an intense bright red color and a characteristic fluorescence in 0.5*N* acetic acid when viewed in the light of an ultraviolet lamp, and readily passes into a deep violet non-crystalline product. Its absorption bands lie in the regions 295-320 mμ and 420-450 mμ (in 2*N* acetic acid).

Chrysopterin. The pigment is encountered in the preparation of the β-barium salt of xanthopterin from the crude product of *Gonepteryx*

[10a] Purrmann, *Annalen*, 546, 98 (1940). *See also* Schopf, *Naturwiss.*, 28, 478 (1940); Wieland. Tartter: *Ibid.*, 543, 287 (1941); Wieland, Tartter, Purrmann: *Ibid.*, 545, 209 (1940). (1933)]; Wrede, Rothaas: *Z. physiol. Chem.*, 226, 95 (1934).

[11] cf. also Schöpf, Becker, Reichert: *Ann.*, 539, 156 (1939).

rhamni as its dark-yellow-brown amorphous barium salt. The free orange yellow pterin was obtained by acidification as a product with the formula $C_{19}H_{16}O_8N_{14}$. Its solution has a blue fluorescence similar to that of erythropterin.

Guanopterin,[11] is distinguished by being more readily soluble in baryta water. It may be purified through its difficultly soluble sulfate which crystallizes well. Guanopterin crystallizes in colorless needles with the probable molecular formula $C_{19}H_{20}O_3N_{20}$.

Mesopterin, $C_{19}H_{17}(O+N)_{24}$, is a weakly basic pterin which has been recognized in the female *Gonepteryx rhamni* and in wasps.

Uropterin.[12] Examination of human urine has led to the separation of fractions from which two yellow fluorescent pigments have been isolated. One of these is present in a dilution of about 1:10 and resembles the pterins in its fluorescent behavior and in giving a positive murexide reaction.

Among other sources of pterins may be mentioned human faeces, ox-liver, rabbit and horse urine, and hay. It is noteworthy also that allantoin,[13] which must have been in some way chemically bound, was found in working up the pigments of *Catopsilia argante* and *Catopsilia rurina*. Xanthine [14] has been isolated from the wings of *Pierides*.

Derivatives of Pyrrole [15]

Prodigiosin.[16] *Bacillus prodigiosus* is frequently to be found in water and the soil. It is not known to have any ill effects but may give rise to flecks resembling drops of blood on foodstuffs, as cultures of the bacillus are red. In former centuries these red specks were sometimes the cause of mistakes, with serious consequencs. It is related [17] that the appearance of such red flecks on sacramental wafers in the middle ages led to the belief "they had been defiled by Jews and were bleeding. The offenders were quickly found and usually burned or simply put to death. Hecatombs of men have met their end through *Prodigiosus*." Prodigiosin has the composition agreeing with $C_{20}H_{25}ON_3$, forms a red, brittle, amorphous mass with a metallic luster and has no sharp melting point but sinters at 70-80°. Its perchlorate on the other hand forms large needles with a metallic luster (m.p. 228°). The pigment does not possess any considerable fastness to light.

Hydrogenation results in the absorption of an amount of hydrogen corresponding to one double linking, and methoxyl determination reveals one methoxyl group. Dry distillation of prodigiosin in a stream of hydrogen at 250° yielded a degradative fragment, $C_{10}H_{17}N$, which was recognized as a pyrrole derivative bearing an α-methyl group but no substituent on the nitrogen atom. As further oxidation yielded *n*-capronic acid, this fragment must be either a β- or β'-*n*-amylpyrrole. That it is in fact the β-derivative was conclusively proved by synthesis from 2-amino-octan-3-one and oxalacetic ester:

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[12] Koschara: *Z. physiol. Chem.*, 240, 127 (1936).

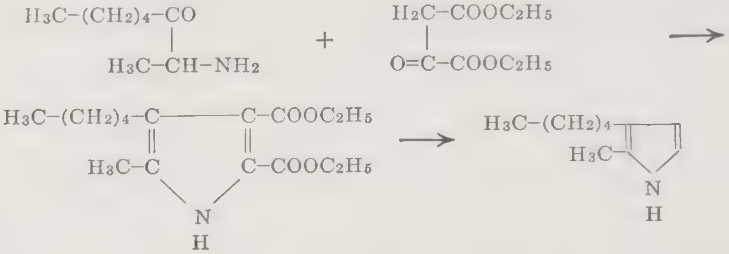
[13] Schöpf, Kottler, Reichert: *Ann.*, 539, 160 (1939).

[14] Purrmann: *Z. physiol. Chem.*, 260, 105 (1939).

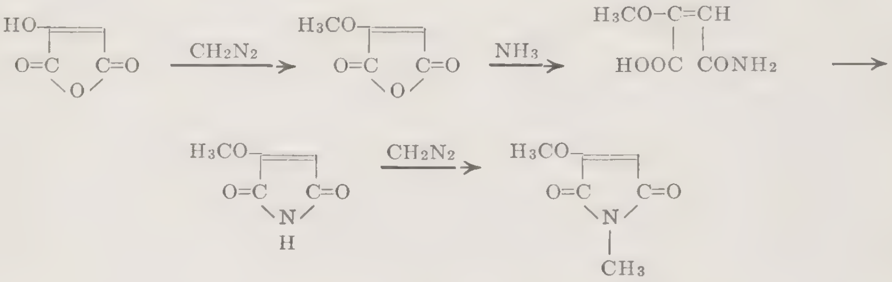
[15] H. Fischer and H. Orth, "Die Chemie des Pyrrol," Vols. **I, II**, Leipzig, 1934.

[16] Wrede, Hettche: *Ber.*, 62, 2678 (1929); Wrede: *Z. physiol. Chem.*, 210, 125 (1932); Wrede, Rothaas: *Z. physiol. Chem.*, 215, 67 (1933); 219, 267 (1933); reply to Raudnitz [*Naturwiss.*, 21, 518 (1933)]; Wrede, Rothaas: *Z. physiol. Chem.*, 226, 95 (1934).

[17] Scheurlen: *Arch. Hygiene*, 26, 3 (1896); an exhaustive summary of the literature is given here.

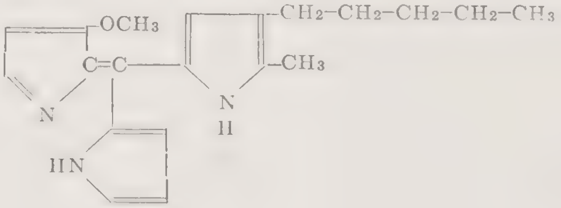

H3C–(CH2)4–CO
H3C–CH–NH2
+
H2C–COOC2H5
O=C–COOC2H5
H3C–(CH2)4–C
C–COOC2H5
H3C–C
C–COOC2H5
N
H
H3C–(CH2)4
H3C
N
H

Oxidation of the pigment with hydrogen peroxide gave, among other products, a compound, $C_6H_7O_3N$, which was shown to be N-methylmethoxymaleimide (I) by comparing it with a synthetic compound of that constitution:


HO
O=C
C=O
O
CH2N2
H3CO
O=C
C=O
O
NH3
H3CO–C=CH
HOOC CONH2
H3CO
O=C
C=O
N
H
CH2N2
H3CO
O=C
C=O
N
CH3

(I)

Complete hydrogenation with palladium on charcoal in acetic acid at 60° results in the uptake of 14 atoms of hydrogen to give a product, oxidation of which in acid solution yielded pyrrolidine, a compound $C_{10}H_{21}N$, oxaminic acid, succinic acid and a compound containing no methoxyl groups but resembling proline. One unsaturated pyrrole ring may thus be deduced and the following formula therefore appears to be correct:


OCH3
C=C
N
HN
–CH2–CH2–CH2–CH2–CH3
–CH3
N
H

Prodigiosin may be obtained from an agar culture to which has been added meat broth, "Peptonum siccum sine sale," dextrose and magnesium sulfate, two square meters of culture surface yielding 0.4 gram of pigment.

In addition to the above a considerable number of bacterial pigments are described in the literature (these are reviewed by Kögl[18]) of which no molecular formulas are known.

Blood pigments. Blood pigments[19] are extraordinarily widely distributed in nature but are distinguished more by associated proteins than by the pigment compounds themselves. Although the differentiation of the protein constituents is even today attended by considerable difficulty, the characteristics of the pigment components, consisting of pyrrole coloring matters, are more readily determined, especially when there are considerable variations in the hemin side-chains.

The coloring matter of human blood consists of hemoglobin which, according to prevailing opinion, is composed of 95 per cent of the protein globin and 5 per cent of pigment. The pigment component was formerly termed "hemochromogen," but according to H. Fischer this term should be reserved for the addition compounds of "hems" with nitrogenous bases. By "hem" is understood the compound $C_{34}H_{32}O_4N_4Fe$, responsible for the color of blood, and also analogous complexes containing divalent iron.

It has been estimated that the blood pigment becomes completely converted into bile pigment within 2½ months, and regeneration must therefore take place in the human organism also in this period. Nothing is yet known of the mechanism of this regeneration process.

The protein-pigment association of hemoglobin may be disrupted, for example by pouring[20] the blood into hot acetic acid saturated with common salt, whereupon hemin, $C_{34}H_{32}O_4N_2FeCl$, a modified product, is obtained. One can now replace the chlorine atom attached to iron by a hydroxyl group to obtain hematin. If the iron is removed from hemin or hem (formerly hemochromogen) a number of porphyrins of somewhat differing compositions are obtained, their fine structure depending on the nature of the acid employed to effect the elimination. They may be reconverted into complex iron salts or, in other words, into hemins.[21]

The determination of the constitution of the blood pigment is dependent, therefore, on a knowledge of the constitution of the porphyrins. Numerous porphyrins[22] are known naturally occurring in the animal, vegetable, and mineral kingdoms,[23] including some which appear in the human organism under pathological conditions. Little is known of their ultimate physiological importance, although they appear to function as sensitisers.[22]

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[18] Kögl in Klein: "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 1436.

[19] Exhaustive account: H. Fischer and H. Orth: "Die Chemie des Pyrrols," Vol. **II,** p. 364.

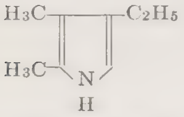
[20] Teichmann: *Z. rat. Med.*, **N.F.** 3, 375 (1853); for earlier work, see the compilative memoir by H. Fischer: *Ber.*, 60, 2611 (1927); H. Fischer: *Naturwiss.*, 18, 1026 (1930); *Angew. Chem.*, 44, 617 (1931).

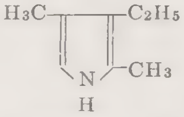
[21] Zaleski: *Z. physiol. Chem.*, 43, 11 (1904).

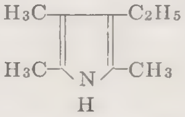
[22] Literature, see H. Fischer: *Ber.*, 60, 2611 (1933), particularly p. 2612.

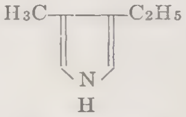
The exceedingly useful reductive degradation of the blood pigment leading to smaller fragments was due to Nencki,[24] and the working up and separation of the mixtures so produced, mixtures which were originally thought to be homogeneous compounds, is linked with the names of Piloty,[25] Willstätter,[26] and H. Fischer.[27] Among the methods which these workers used to effect the isolation of individual compounds were fractional salt formation with picric acid, fractional crystallization of the picrates, methods based on varying behavior on coupling with diazobenzene sulfonic acid and varying basicity of the fission products, and distillation of esters where the degradation products were acidic. By such methods the following fission products were obtained:

1. Pyrrole bases

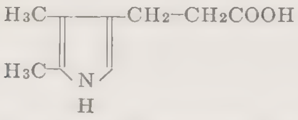

Hemopyrrole

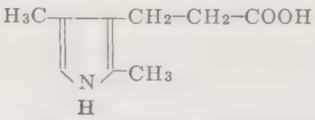

Kryptopyrolle

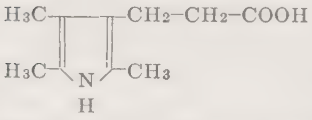

Phyllopyrolle

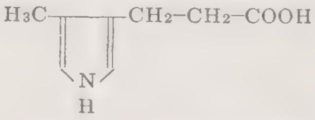

Opsopyrolle

2. Pyrrole acids


Hemopyrrole carboxylic acid


Kryptopyrrole carboxylic acid


Phyllopyrrole carboxylic acid


Opsopyrrole carboxylic acid

The pyrrole carboxylic acids carry a propionic acid residue in place of the ethyl group of the pyrrole bases. Again, oxidative degradation of hemin and the porphyrins and oxidation of the original mixture of hemopyrrole compounds, first carried out by Küster,[28] gave rise to the following pyrrole compounds:

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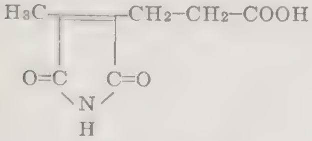
[23] Porphyrins derived from chlorophyll have been detected in coals and other minerals and the fact that they are not stable above moderate temperatures has stimulated geological thought: Treibs: *Ann.*, **509**, 103 (1934); **510**, 42 (1934); **517**, 172 (1935); **520**, 144 (1935); comprehensive account: *Angew. Chem.*, **49**, 682 (1936).

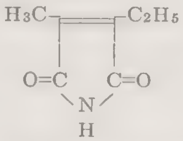
[24] Earlier literature: Hahn: *Z. Biol.*, **64**, 141 (1914).

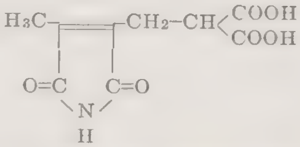
[25] cf. Piloty: *Ann.*, **366**, 237 (1909); Piloty, Dormann: *Ber.*, **45**, 2592 (1912).

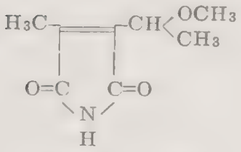
[26] R. Willstätter and A. Stoll: "Untersuchungen über Chlorophyll," Chapter 22.

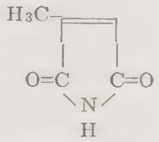
[27] H. Fischer, Eismayer: *Ber.*, **47**, 1820 (1914); H. Fischer, Bartholomäus: *Ber.*, **45**, 467, 1315 (1912); H. Fischer, Röse: *Ber.*, **47**, 791 (1914); *Z. physiol. Chem.*, **87**, 39 (1913).

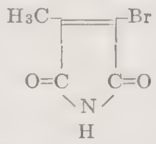

Hematic acid


Methylethylmaleimide

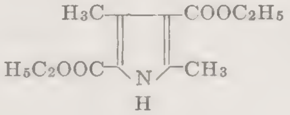

Carboxylated hematic acid


Imide (m.p. 64°)


Citraconimide


Bromcitraconimide

Of these, hematic acid [29] has been synthesized and methylethylmaleimide is obtained by decarboxylation; similarly the carboxylated hematic acid may be decarboxylated to hematic acid [30]; the constitution [31] of the imide (m.p. 64°) has been determined and the known citraconimide [32] and bromcitraconimide [33] complete the series. Any doubt of the constitution of the products of reductive degradation, the hemopyrrole bases, has been removed by their synthesis [34]; the synthetic methods were usually modelled on the acetoacetic ester synthesis of Knorr, leading to dimethyldicarbethoxypyrrole [35] (I).


(I)

It may be concluded, from the degradation of hemin and its derivatives to such fragments, that pyrrole compounds are the units in the hemin molecule and in the porphyrins. Hemin is characterized as a dicarboxylic acid and a determination of its molecular weight [36] indicates the presence of four pyrrole nuclei, a similar deduction attaching to the porphyrins; this view is substantiated by the analytical fact that all porphyrins contain four atoms of nitrogen. Among the physiologically and structurally most important porphyrins are:

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[28] Küster: *Ber.*, **35,** 2948 (1902); **40,** 2017 (1907).

[29] Küster, Weller: *Ber.*, **47,** 532 (1914).

[30] Constitution, cf. H. Fischer: *Ber.*, **60,** 2611 (1927) and particularly p. 2615.

[31] Küster: *Z. physiol. Chem.*, **163,** 270 (1927); Küster, Schlayer: *Ibid.*, **168,** 295 (1927); H. Fischer, Treibs, Hummel: *Ibid.*, **185,** 33 (1929).

[32] H. Fischer, Lindner: *Ibid.*, **161,** 18 (1926).

[33] H. Fischer, Kotter: *Ber.*, **60,** 1862 (1927).

[34] Hemopyrrole: Piloty, Blömer: *Ber.*, **45,** 3749 (1912); kryptopyrrole: Knorr, Hess: *Ber.*, **44,** 2758 (1911); H. Fischer, Schubert: *Ber.*, **56,** 1202 (1923); **57,** 610 (1924); phyllopyrrole: Willstätter, Asahina: **385,** 188 (1911); H. Fischer, Bartholomäus: *Ber.*, **45,** 466 (1912); opsopyrrole: H. Fischer, Halbig: *Ann.*, **450,** 151 (1926); H. Fischer, Sturm, Friedrich: *Ann.*, **461,** 244 (1928); hemopyrrole carboxylic acid: H. Fischer, Treibs: *Ber.*, **60,** 377 (1927); kryptopyrrole carboxylic acid: H. Fischer, Weiss: *Ber.*, **57,** 602 (1924); phyllopyrrole carboxylic acid: H. Fischer, Nenitzescu: *Ann.*, **439,** 175 (1924); opsopyrrole carboxylic acid: H. Fischer, Treibs: *Ber.*, **60,** 377 (1927); H. Fischer, Lamatsch: *Ann.*, **462,** 240 (1928); cf. H. Fischer: *Ber.*, **60,** 2611 (1927) and especially p. 2619.

[35] Details in H. Fischer: *Ber.*, **60,** 2611 (1927) and especially pp. 2619 and 2620 (table).

[36] H. Fischer, Hahn: *Ber.*, **46,** 2308 (1913).

Aetioporphyrin $C_{32}H_{38}N_4$
Deuteroporphyrin $C_{30}H_{30}O_4N_4$
Protoporphyrin $C_{34}H_{32}O_4N_4$
(=Oöporphyrin
=Kämmerer's porphyrin)
Haematoporphyrin $C_{34}H_{36}O_6N_4$
Mesoporphyrin $C_{34}H_{38}O_4N_4$
Coproporphyrin $C_{36}H_{38}O_8N_4$
Conchoporphyrin $C_{37}H_{38}O_{10}N_4$
Uroporphyrin $C_{40}H_{38}O_{16}N_4$

Many porphyrins have been prepared synthetically but many of the individual members of the above series are more readily obtained from natural sources:

Mesoporphyrin [37] is obtainable from hemin by treating with acetic and hydriodic acids.

Hematoporphyrin [38] may be prepared from hemin with acetic and hypobromic acids.

Protoporphyrin [39] is produced on pouring blood into concentrated hydrochloric acid, or more satisfactorily by the action of iron and formic acid on hemin.[40] It is identical with Kämmerer's porphyrin obtained by the action of bacteria on the blood pigment [41] and with oöporphyrin,[42] the pigment of the flecked eggshells of the plover.

Uroporphyrin [43] and **coproporphyrin** [44] are excretory products appearing in the human organism under pathological conditions, although uroporphyrin is also found as its copper salt (turacin) in the quill-feathers of turakao birds [45] and in the shells of the mussel *Pteria vulgaris*,[46] *Pteria radiator* [47] containing conchoporphyrin. Finally, deuteroporphyrin [48] may be obtained from the corresponding hemin and is formed by the putrefaction of blood under alkaline conditions. The term *aetioporphyrin* [49] comprises a number of porphyrins which are free from carboxyl groups, and the constitution of these will be discussed later.

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[37] Nencki, Zaleski: *Ber.*, **34**, 997 (1901).

[38] Nencki, Sieber: *Arch. exp. Path. Pharmakol.*, **24**, 430 (1888).

[39] H. Fischer, Schneller: *Z. physiol. Chem.*, **130**, 302 (1923).

[40] H. Fischer, Pützer: *Ibid.*, **154**, 39 (1926).

[41] Kämmerer: *Deut. Arch. klin. Med.*, **145**, 257 (1924).

[42] H. Fischer, Kögl: *Z. physiol. Chem.*, **131**, 241 (1923); **138**, 262 (1924); H. Fischer, Lindner: *Ibid.*, **142**, 141 (1925).

[43] H. Fischer: *Ber.*, **60**, 2611 (1927) and especially p. 2626; H. Fischer, Hofmann: *Z. physiol. Chem.*, **246**, 15 (1937); H. Fischer, Müller: *Ibid.*, **246**, 31 (1937).

[44] H. Fischer: *Ber.*, **60**, 2611 (1927); p. 2627; Zeile, Rau: *Z. physiol. Chem.*, **250**, 197 (1937); Mertens: *Ibid.*, **250**, 57 (1937); cf. Waldenström, Fink, Hoerburger: *Ibid.*, **233**, 1 (1935); H. Fischer, Libowitzky: *Ibid.*, **241**, 220 (1936); Watson: *J. Clin. Investigation*, **15**, 327 (1936); **16**, 383 (1937); Rimington: *Z. physiol. Chem.*, **259**, 45 (1939).

[45] H. Fischer, Hilger: *Z. physiol. Chem.*, **138**, 49 (1924); Völker: *Ibid.*, **258**, 1 (1939).

[46] H. Fischer, Haarer: *Ibid.*, **204**, 101 (1932).

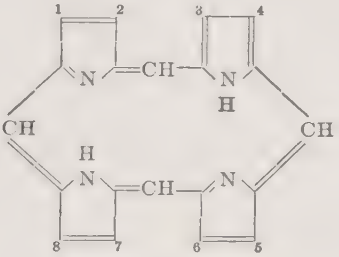
[47] H. Fischer, Jordan: *Ibid.*, **190**, 75 (1930).

[48] H. Fischer, Lindner: *Ibid.*, **161**, 18 (1926).

[49] Willstätter, M. Fischer: *Ibid.*, **87**, 423 (1913); H. Fischer, Kirstahler: *Ibid.*, **198**, 43 (1931).

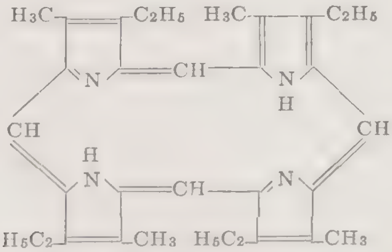
The properties of the porphyrins, their molecular formulas which were analytically confirmed, and the advancing knowledge of the chemistry of their fission products led to the suggestion of a number of constitutional formulas as working hypotheses; and it is noteworthy that of these formulas, that proposed for hemin by Küster[50] is now known to be, with but one slight modification, correct. Today the formulas of hemin and other compounds as porphyrins have been established beyond reasonable doubt by synthesis, and these investigations have provided also a basis for more recent developments in the chemistry of chlorophyll.

The parent compound of the whole group under discussion is porphin (I). It is more difficultly accessible than many of the substituted derivatives, but was synthesized[51] from α-pyrrole aldehyde and later from pyrrole and formaldehyde.



(I)

It should be realized that porphin and its derivatives, like the remarkable synthetic pigments, the phthalocyanines, of Linstead[52] and his co-workers, contain a molecular system including a 16-membered ring of a unique type. The system of double linkages is completely conjugated in both series and their remarkable stability (the natural porphyrins undergo relatively minor changes due to reactive side-chains but the ring system is astonishingly resistant to modification) indicates that the struc-



Aetioporphyrin I

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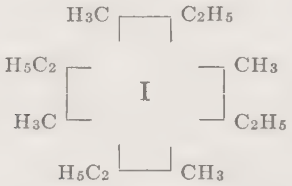
[50] Küster: *Ibid.*, 82, 463 (1912).

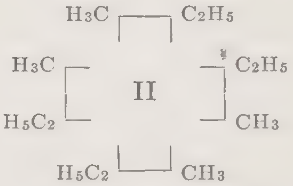
[51] H. Fischer, Gleim: *Ann.*, 521, 157 (1935); Rothemund: *J. Am. Chem. Soc.*, 57, 2010 (1935); 58, 625 (1936).

[52] Linstead: *Ber.*, 72A, 93 (1939).

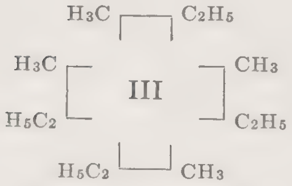
tures are in reality resonance hybrids. The formula given above is but one of a number of structures which differ only in the disposition of their double-bonds; they therefore do not enjoy separate existences, just as the Kekulé forms of benzene cannot be individually isolated. Thus no structural significance is to be placed on this mode of representing the imino hydrogen atoms on adjacent nitrogen atoms, and indeed the valences may be rearranged to accommodate them on diagonal nitrogen atoms. It has been suggested that, in the synthesis of unsubstituted porphin from pyrrole and formaldehyde, a second pigment which arises differs from the first only in the ortho and para arrangement of the imino groups. The divergence in properties, however, is surprisingly large if this is the true interpretation. It will be appreciated that all the β-positions of the pyrrole nuclei are identical, and thus only four different aetioporphyrins (*i.e.*, porphyrins derived from four β-methyl-β′-ethylpyrrole nuclei) may be derived from porphyrin. That given above is designated by H. Fischer as aetioporphyrin I, and the formulas of all four isomerides are given in the following abbreviated forms, preferred by some workers, where the strokes represent β-β′-bonds of the pyrrole nuclei.*



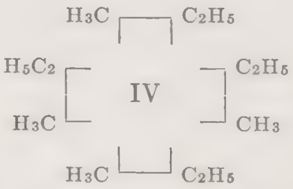
1,3,5,7-Tetramethyl-2,4,6,8-tetraethylporphin



1,4,5,8-Tetramethyl-2,3,6,7-tetraethylporphin



1,3,5,8-Tetramethyl-2,4,6,7-Tetraethylporphin



1,4,6,7-Tetramethyl-2,3,5,8-Tetraethylporphin

These have all been synthesized. As was earlier anticipated, hemin[53] has the structure:

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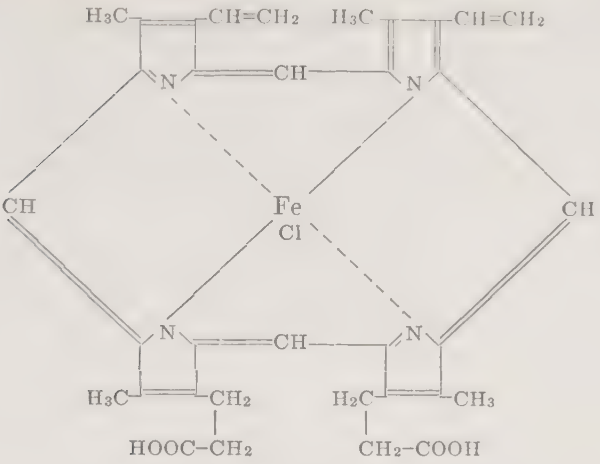
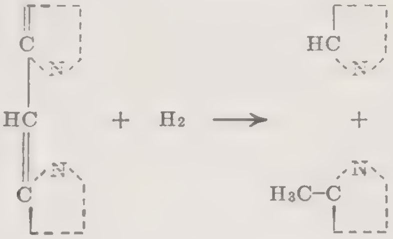
* For metal complexes and their potentiometric titration see Mansfield Clark, Taylor, Davies, Vestling, Perkins, *J. biol. Chem.*, 135, 543 (1940).

[53] Earlier formulas which are of only historical interest: Nencki: Zaleski: *Ber.*, 34, 997 (1901); Piloty, Dormann: *Ann.*, 388, 313 (1912); Piloty, Stock: *Ibid.*, 392, 215 (1912); Willstätter: *Ber.*, 47, 2861 (1914).



It is thus derived from aetioporphyrin III.

As developed above, the porphyrin formulas may be regarded as a linking of four pyrrole rings, or more formally considered, one pyrrole, one maleimide, and two pyrrolenine rings, by four methine bridges, the closed conjugated system being responsible for the color. Reductive fission at the methine bridges may now be interpreted as in (I) so that a mixture of pyrroles is obtained. In the oxidative fission it is again the methine groups which are attacked, and hematic acid is obtained in a yield corresponding to the two pyrrole nuclei bearing propionic acid residues, but those bearing vinyl groups are apparently completely oxidized. Again, the reaction with hydroiodic acid, leading to mesoporphyrin, is clearly a reduction of the two vinyl groups to ethyl groups as is indicated by subsequent oxidation; attack at the vinyl groups is no longer possible, and methylethylmaleimide and two molecules of hematic acid are obtained. The presence of the vinyl groups may also be detected by the addition of diazoacetic ester.[54]



(I)

Protoporphyrin is known chiefly as hemin from which the iron has been eliminated and may thus be reconverted into hemin. Haematoporphyrin, which is formed when hydrogen bromide is used to eliminate the iron, is produced by virtue of the following reactions of the vinyl groups:

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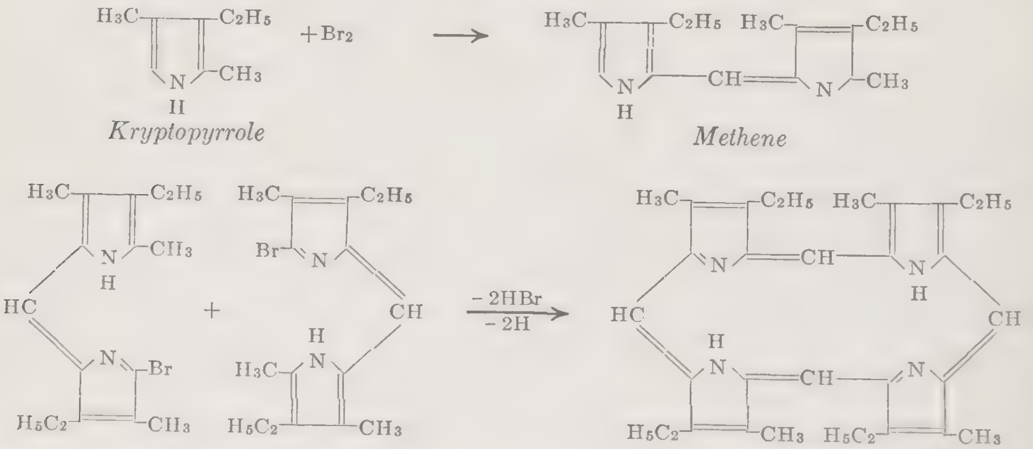
[54] H. Fischer, Medick: *Ann.*, **517**, 246 (1935).

$$-CH{=}CH_2 \longrightarrow CHBr-CH_3 \longrightarrow -CHOH-CH_3$$

This is reflected in the oxidation of hematoporphyrin when the nuclei bearing the hydroxyethyl groups are destroyed, whereas, if these groups are first methylated, degradation yields the imide (m.p. 64°).

Deuteroporphorin, apart from its lack of iron, is distinguished from hemin by containing no vinyl groups. Direct oxidation thus yields citraconimide, whereas dibromdeuteroporphyrin and bromcitraconimide are obtained by bromination and subsequent oxidation. On oxidation, uroporphyrin gives carboxylated hematic acid, and coproporphyrin gives hematic acid itself and by reduction, hemopyrrole carboxylic acid; no basic products are found. The conclusion may therefore be drawn that coproporphyrin is 1,3,5,7-tetramethylporphin-2,4,6,8-tetrapropionic acid, *i.e.*, aetioporphyrin I, in which all the ethyl groups are replaced by propionic residues, whereas uroporphyrin probably contains succinic acid residues. Aetioporphyrin I is formed by decarboxylation. It has already been mentioned that conchoporphyrin contains the same structure, as it has but one more carboxyl group than coproporphyrin.

These conceptions have been verified by syntheses of porphyrins extending to that of hemin. The action of bromine [55] on kryptopyrrole leads to a dipyrrylmethene, containing a free α-position which is further replaceable by bromine. Such a bromine atom is only loosely bound, and a substituted methene of this kind yields an aetioporphyrin on treatment with acids [56] (*e.g.*, formic acid):



Kryptopyrrole

Methene

Aetioporphyrin I

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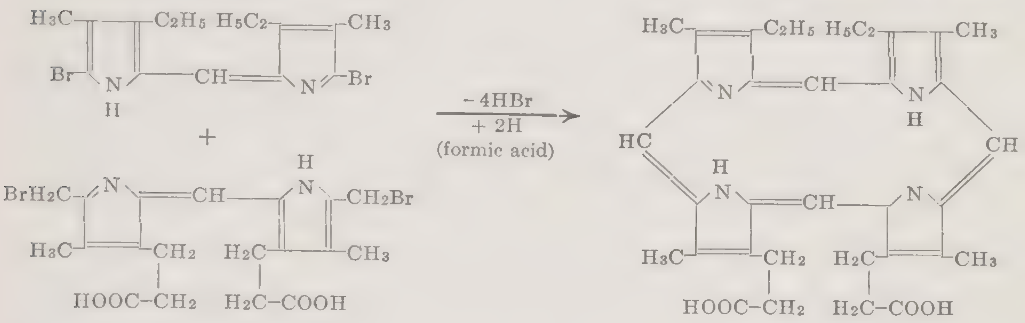
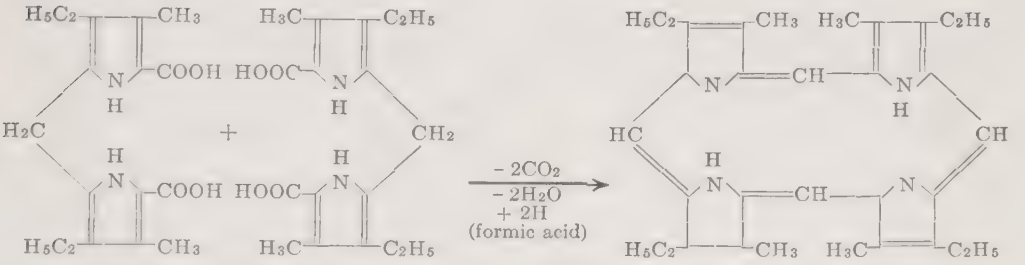
[55] H. Fischer: *Sitzber. Acad. Wiss. München, Math.-Physik. Kl.*, **1915**, 401; H. Fischer, Scheyer: *Ann.*, **434**, 237 (1923); H. Fischer, Halbig: *Ann.*, **447**, 123 (1926); H. Fischer, Ernst: *Ann.*, **447**, 139 (1926).

[56] H. Fischer, Klarer: *Ann.*, **448**, 178 (1926). Graphically described by H. Fischer: *Ber.*, **60**, 2611 (1927), particularly p. 2637: "Es herrschte eine fieberhafte Erregung im Laboratorium bei welchem Doktoranden wohl zuerst Porphyrinbildung eintreten würde." (Feverish excitement reigned in the laboratory as to which student would be the first to induce the formation of porphyrin.)

As is obvious from the formula, the synthesis involves a dehydrogenation.[57] This synthesis of porphyrins by condensation of two molecules of simple nuclear brominated methenes has been applied to the condensation of different methenes and also to syntheses in which one of the two methenes can carry a free α-methine group instead of bromine; it is by such means that the synthesis of porphyrin monopropionic acids has been effected. There are also available doubly brominated dipyrrylmethenes which are of particular value in syntheses of the following kind:


H₃C
C₂H₅
H₅C₂
CH₃
Br
N
H
CH
N
Br
+
BrH₂C
N
CH
H
N
CH₂Br
H₃C
CH₂
H₂C
CH₃
HOOC–CH₂
H₂C–COOH
– 4HBr
+ 2H
(formic acid)
H₃C
C₂H₅
H₅C₂
CH₃
N
CH
N
H
HC
CH
H
N
CH
N
H₃C
CH₂
H₂C
CH₃
HOOC–CH₂
H₂C–COOH

Syntheses of the porphyrin nucleus can also be carried out starting with carboxylic acids [58]:


H₅C₂
CH₃
H₃C
C₂H₅
N
H
COOH
HOOC
N
H
H₂C
+
CH₂
H
N
COOH
HOOC
H
N
H₅C₂
CH₃
H₃C
C₂H₅
– 2CO₂
– 2H₂O
+ 2H
(formic acid)
H₅C₂
CH₃
H₃C
C₂H₅
N
CH
N
H
HC
CH
H
N
CH
N
H₅C₂
CH₃
H₃C
C₂H₅

Dikryptopyrrylmethane dicarboxylic acid

Aetioporphyrin II (Isoaetioporphyrin)

With such methods a number of porphyrins [59] have been obtained synthetically. It may be mentioned in conclusion that a mixture of two isomeric aetioporphyrins is obtained from opsopyrrole, formaldehyde, and formic acid or chloromethyl ether.[60]

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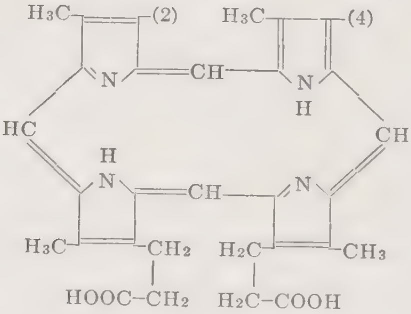
[57] Discussion of the reaction mechanism: H. Fischer: *Ber.*, **60**, 2611 (1927) and p. 2628.

[58] H. Fischer, Halbig: *Ann.*, **448**, 193 (1926); H. Fischer, Walach: *Ann.*, **450**, 164 (1926); H. Fischer, Andersag: *Ann.*, **450**, 201 (1926).

[59] Syntheses of porphyrins: H. Fischer, Andersag: *Ann.*, **458**, 117 (1927); H. Fischer, Treibs: *Ann.*, **450**, 132 (1926); *Ber.*, **60**, 377 (1927); H. Fischer, Heisel: *Ann.*, **457**, 83 (1927); H. Fischer, Lindner: *Z. physiol. Chem.*, **161**, 1 (1926); H. Fischer, Halbig, Walach: *Ann.*, **452**, 268 (1927); H. Fischer, Stangler: *Ann.*, **459**, 53 (1927); **462**, 251 (1928); H. Fischer, Friedrich, Lamatsch, Morgenroth: *Ann.*, **466**, 147 (1928); H. Fischer, Kirstahler: *Ann.*, **466**, 178 (1928); H. Fischer, Lamatsch: *Ann.*, **462**, 240 (1928); H. Fischer, Bäumler: *Ann.*, **468**, 58 (1929); H. Fischer, Weichmann, Zeile: *Ann.*, **475**, 241 (1929); H. Fischer, Kirrmann: *Ann.*, **475**, 266 (1929); H. Fischer, Platz, Morgenroth: *Z. physiol. Chem.*, **182**, 265 (1929); H. Fischer, Jordan: *Ibid.*, **191**, 36 (1930); H. Fischer, Schormüller: *Ann.*, **482**, 232 (1930); H. Fischer, Siebert: *Ann.*, **483**, 1 (1930); H. Fischer, Goldschmidt, Nüssler: *Ann.*, **486**, 1 (1931); H. Fischer, Nüssler: *Ann.*, **491**, 162 (1931); H. Fischer, Hierneis: *Z. physiol. Chem.*, **196**, 155 (1931); H. Fischer, Kirstahler, v. Zychlinski: *Ann.*, **500**, 1 (1932); H. Fischer, Riedl: *Z. physiol. Chem.*, **207**, 193 (1932); H. Fischer, Bertl: *Ibid.*, **229**, 37 (1934); H. Fischer, Haarer: *Ibid.*, **229**, 55 (1934); H. Fischer, von Holt: *Ibid.*, **227**, 124 (1934); **229**, 93 (1934).

As illustrated above with aetioporphyrin, work in this field is complicated and is rendered more laborious by the ever-present possibility of isomerism. Thus four structural isomerides are possible even with only two different β-substituents; this is exactly the case with the coproporphyrins with four methyl groups and four propionic residues; and of the four isomerides, two have been identified as those occurring naturally in cases of pathological metabolic derangements (porphyrinuria).

The order of the substituents in hemin has been established by synthesis of the corresponding mesoporphyrins.[61] Here 15 isomerides were possible by replacing two ethyl groups in each of the four aetioporphyrins by propionic acid groups. In this way mesoporphyrin from natural sources was found to be identical with 1,3,5,8-tetramethyl-2,4-diethylporphin-6,7-dipropionic acid, and is thus structurally related to aetioporphyrin III. This result opened a route to the synthesis of hemin[62] itself by first preparing deuteroporphyrin (A), 1,3,5,8-tetramethylporphin-6,7-dipropionic acid. Iron was then introduced, and the resulting deuterohemin was then acetylated[63] to provide 2,4-diacetyldeuterohemin when the iron was again eliminated. Diacetyldeuteroporphyrin so formed was reduced to dihydroxyethyldeuteroporphyrin, *i.e.*, hematoporphyrin, from which two molecules of water were removed, giving protoporphyrin with two vinyl groups. Finally, iron was once more introduced to give a compound identical with natural hemin.



(A)

This synthesis, it will be noted, is a complete one;[64] and since the pyrrole units were built up from acetoacetic ester which, as the pathological metabolism of diabetics shows, is not foreign to the living organism, it may be inquired whether the organism itself does not synthesize the blood pigment in some such way.

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[60] H. Fischer, Sturm, Friedrich: *Ann.*, **461**, 244 (1928).

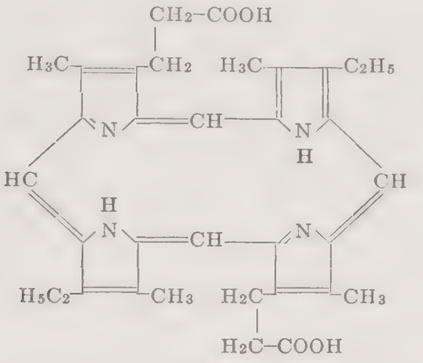
[61] H. Fischer, Stangler: *Ann.*, **459**, 53 (1927); in all, 12 mesoporphyrins have been synthesized.

[62] H. Fischer, Zeile: *Ann.*, **468**, 98 (1929).

[63] Only the iron salt allows the introduction of acetyl groups.

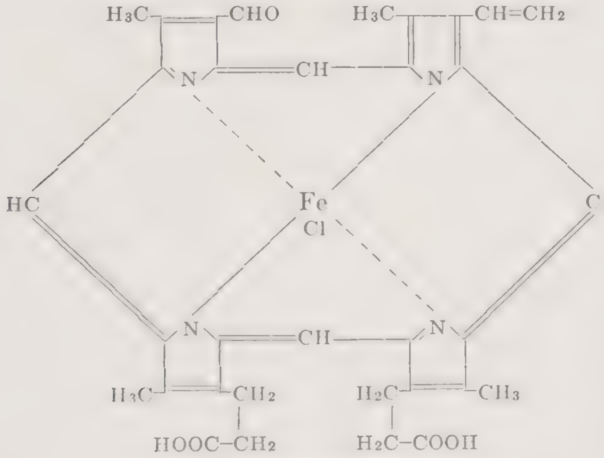
[64] No attempt has been made to include details of this and other syntheses, but references to the literature have been carefully collected. The porphyrins are distinguished by their characteristic spectra. For hemin formula cf. also Stern, Wenderlein: *Z. physic. Chem. Abt. A*, **175**, 429 (1936); Lindenfeld: *Rosziki Chemji*, **15**, 516 (1935); *Chem. Zentr.*, **1936**, *I*, 2363.

More recent work appears to countenance the hypothesis that the blood pigment contains an isomeride[65] of hemin in addition to hemin itself which would account for the somewhat indistinct melting points of "analytical" porphyrins prepared from it. It is suggested that the isomeride is derived from mesoporphyrin (II):


(II)

and a similar hypothesis is advanced for the bile pigment and chlorophyll.

Spirographis hemin. This pigment is the prosthetic group of the blood pigment, chlorocruorin, of a number of worms (*Sabella, Serpula, Spirographis*) and snails (*Siphonostoma*). It was first obtained in crystalline form by Fox[66] and examined by Warburg,[67] but its constitution


Aetioporphyrin
(III)

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[65] H. Fischer: *Z. physiol. Chem.*, **259**, I and 96 (1939).
[66] Fox: *Proc. Roy. Soc. London (B)*, **99**, 199 (1926).
[67] Warburg, Negelein, Haas: *Biochem. Z.*, **227**, 171 (1930); Warburg, Negelein: *Ibid.*, **244**, 9, 239 (1932).

was elucidated by H. Fischer,[68] when the experience gained and methods elaborated in attacking the problem of hemin were of real service. Spirographis hemin, it is seen, is also derived from aetioporphyrin III.

260 mg of the dimethyl ester of spirographis hemin were obtained from 10.5 kg of the worms.

Cytochrome.[69] MacMunn in 1886 had remarked on the presence of a pigment with a four-banded absorption spectrum resembling that of hemochromogen in vigorously respiring cells. Our knowledge of "myohematin" was not advanced until 1925, when it was rediscovered by Keilin, who gave it the name *cytochrome* and demonstrated its almost universal distribution in nature. Thus cytochrome has been found in a large number of higher and lower animals, molluscs, crustacea, insects, and bacteria and in plant tissues, such as potatoes, cereals, yeast, flower stamens, etc. Cytochrome can be reversibly oxidized and reduced; in the latter condition the four bands in the spectrum are due not to one compound, but to a complex of three hems or hemochromogens, as they would formerly have been termed.

Of these compounds, cytochrome *c*[70] is believed to be a derivative of aetioporphyrin III; on elimination of iron, it gives porphyrin *c*, $C_{45}H_{54}O_9N_6S_2$, on the basis of which a partial formula for cytochrome *c* has been suggested.

The ability of cytochrome to undergo reduction and reoxidation is responsible for its reaction with a number of dehydrogenase enzymes and its function as a respiratory carrier. It has been estimated from spectroscopic data that all of the respiration of baker's yeast proceeds by virtue of the carrier action of cytochrome; and although all the interrelationships are still far from being clearly understood, it is certain that cytochrome plays an important role in many cells in assisting the transference of hydrogen from a substrate to molecular oxygen.

Cytochrome shows absorption bands in the reduced state at 601.9, 566,5-550.2-521 mμ. In the oxidized form faint bands are observed at 520-540-550-570 mμ.

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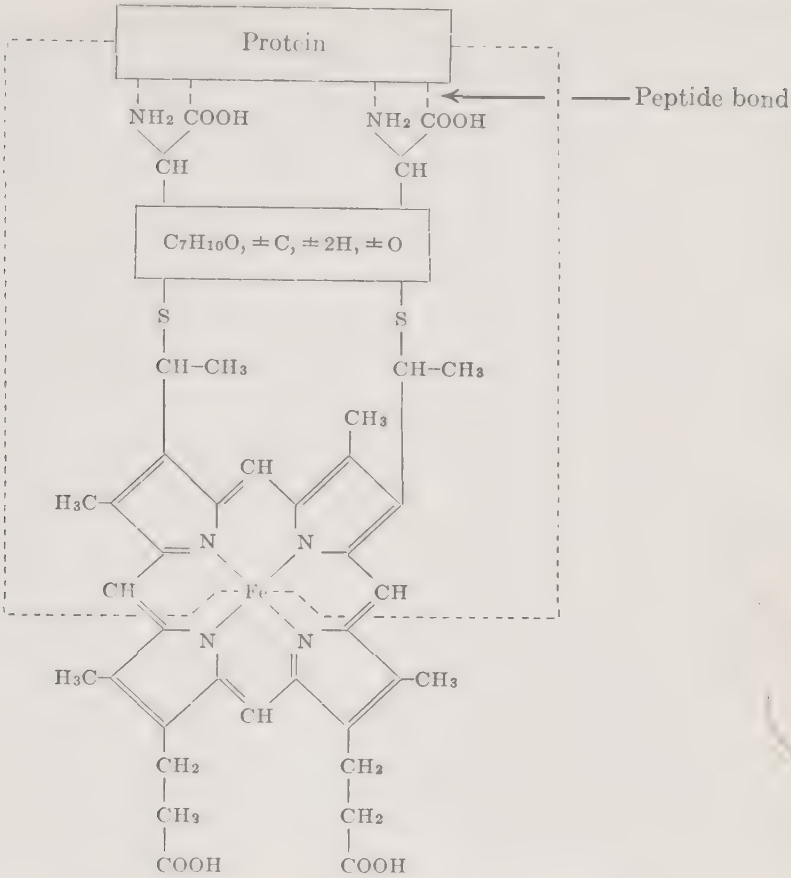
[68] H. Fischer, v. Seemann: *Z. physiol. Chem.*, **242**, 133 (1936); the earlier literature is given here, together with a selection of pigments the inclusion of which among those of the pyrrole group remains uncertain. H. Fischer, v. Seemann: *Angew. Chem.*, **49**, 461 (1936); Keilin, Hartree: *Proc. Roy. Soc. London (B)*, **122**, 298 (1937); Theorell: *Chem. Zentr.*, **1938**, I, 3209.

[69] Keilin: *Proc. Roy Soc. London (B)*, **98**, 312 (1925); H. Fischer, Hilmer: *Z. physiol. Chem.*, **153**, 167 (1926) and p. 195; v. Euler, Fink: *Z. physiol. Chem.*, **164**, 69 (1927); H. Fischer, Schwerdtel: *Ibid.*, **175**, 248 (1928); Bierich, Rosenbohm: *Ibid.*, **184**, 246 (1929); v. Euler, Hellström: *Ibid.*, **190**, 189 (1930); Hill, Keilin: *Proc. Roy. Soc. London (B)*, **107**, 286 (1930); Zeile: *Z. physiol. Chem.*, **207**, 35 (1932); Zeile, Piutti: *Ibid.*, **218**, 52 (1933); Zeile, Reuter: *Ibid.*, **221**, 101 (1933); Zeile: *Ibid.*, **236**, 212 (1935); Dixon, Hill, Keilin: *Proc. Roy. Soc. London (B)*, **109**, 29 (1931); Roche, Bénévent: *Compt. rend.*, **203**, 128 (1936); *Bull. Soc. Chim. Biol.*, **17**, 1473 (1935); **18**, 1650 (1936); Theorell: *Biochem. Z.*, **285**, 207 (1936); Barkau, Schales: *Z. physiol. Chem.*, **246**, 181 (1937); **248**, 96 (1937); Schales: *Ber.*, **70**, 1874 (1937); Yakushiji, Mori: *Acta phytochimica*, **10**, 113 (1937); Katagiri, Masuda, Himemoto: *Chem. Zentr.*, **1937**, I, 4646; **1937**, II, 409 (provisional claim to have synthesized cytochrom *c*). Cytochrome review: Keilin: "Ergebnisse der Enzymforschung," **2**, 239 (1933). Biological significance: Burris in "Respiratory Enzymes," by Elvehjem and Wilson, 1939.

[70] Theorell: *Biochem. Z.*, **298**, 242 (1938). *See also* Zeile, Meyer: *Z. physiol. Chem.*, **262**, 178 (1939); Keilin, Hartree, *Proc. Roy. Soc. London*, **127B**, 167 (1940).


Protein
Peptide bond
NH2 COOH
NH2 COOH
CH
CH
C7H10O, ± C, ± 2H, ± O
S
S
CH–CH3
CH–CH3
CH3
CH
H3C
N
N
CH
Fe
CH
N
N
H3C
CH3
CH
CH2
CH2
CH2
CH2
COOH
COOH

A compound [71] very closely related to, if not identical with, hemin forms the prosthetic group of the enzyme catalase. When associated with its specific protein it is responsible for decomposing hydrogen peroxide formed in the respiring cell. Some peroxidase enzymes also are believed to embody a prosthetic group of the hemin type.

Bilirubin, the biological degradation product of the blood pigment, exists in the bile, probably bound to protein, and is also found in urine. It is best obtained from ox gall-stones. The conversion of the blood pigment into bile pigment with elimination of iron is one of the functions of the liver. By contrast with hemin, bilirubin shows no characteristic spectrum, but on the other hand, is easily recognized by the typical Gmelin color reaction which is an oxidation with nitrous acid, but the precise mechanism of which is still stimulating research workers (see below).

Bilirubin [72] has the formula $C_{33}H_{36}O_6N_4$, thus containing one carbon atom less than hemin. On energetic oxidation [73] it gives only hematic acid (I) and no non-acidic imides, and reduction [74] yields kryptopyrrole (II):

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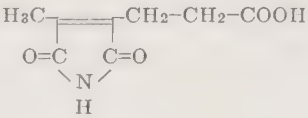
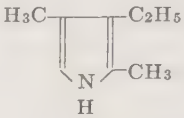
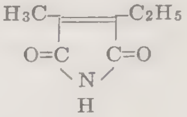
[71] Zeile, Hellström: *Z. physiol. Chem.*, **192**, 171 (1930); Zeile: *Ibid.*, **195**, 39 (1931); Stern: *Nature*, **136**, 302 (1935); *J. Biol. Chem.*, **112**, 661 (1936). The complete literature is given.

[72] Summary of earlier literature: Abderhalden: "Biochemisches Handlexikon," **VI**, 277; **IX**, 388, 407; **X**, 383, 919; **XIV**, 776. Bile pigments, review: Siedel: *Fortschr. Chem. org. Naturstoffe*, 3, 81 (1939).

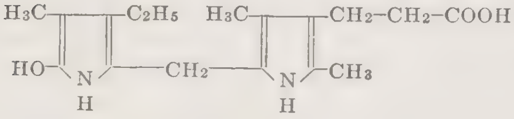

(I)

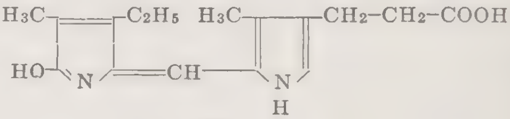

(II)

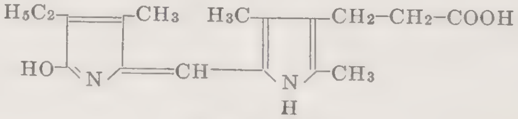

(III)

Reduction may also be effected in smaller steps. Thus catalytic reduction leads to mesobilirubin,[75] $C_{33}H_{40}O_6N_4$, whereas the action of sodium amalgam gives first a colorless intermediate product dihydromesobilirubin,[76] $C_{33}H_{42}O_6N_4$, and then mesobilirubinogen, $C_{33}H_{44}O_6N_4$. The latter formerly bore the name *hemibilirubin,* and is identical with *urobilinogen* occurring in urine and from which the urine pigment urobilin is formed by atmospheric oxidation. Mesobilirubin, like mesobilirubinogen [77] gives methylethylmaleimide (III) in addition to hematic acid.

Energetic reduction of bilirubin gives, in addition to a little kryptopyrrole and kryptopyrrole carboxylic acid, bilirubic acid (IV) [78] as the main product; xanthobilirubic acid (V) [79] is the pyrromethene dehydrogenation product of bilirubic acid. It was later demonstrated that this so-called analytical xanthobilirubic acid [80] obtained from bilirubin and its derivatives is in reality a mixture of the above acid and an isoxanthobilirubic acid [81] (I) (see the unsymmetrical formula of bilirubin):


(IV)


m.p. 287
(V)


(m.p. 289°)
(I)

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[73] Küster: *Ber.*, **30,** 1831 (1897); *Z. physiol. Chem.*, **59,** 63 (1909).

[74] H. Fischer, Röse: *Ber.*, **45,** 3274 (1912).

[75] H. Fischer: *Ber.*, **47,** 2330 (1914); *Z. Biol.*, **65,** 163 (1915).

[76] H. Fischer, Baumgartner: *Z. physiol. Chem.*, **216,** 260 (1933).

[77] H. Fischer: *Ibid.*, **73,** 204 (1911); H. Fischer, Meyer-Betz: *Ibid.*, **75,** 232 (1911); H. Fischer, Meyer: *Ibid.*, **75,** 339 (1911); absorption spectrum of bilirubin: Henry-Cornet, Henry: *Chem. Zentr.*, **1936,** II, 2921; preparation of bilirubin from bile; U. S. Patent 2,049.134 (Petermann), *Chem. Zentr.*, **1937,** I, 2025; supposed isomeride: H. Fischer: *Z. physiol. Chem.*, **259,** I (1939).

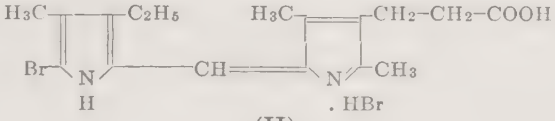
[78] H. Fischer, Röse: *Ber.*, **45,** 1579 (1912); *Z. physiol. Chem.*, **82,** 391 (1912); Piloty, Thannhäuser: *Ann.*, **390,** 191 (1912).

[79] H. Fischer, Röse: *Ber.*, **46,** 439 (1913); Piloty: *Ber.*, **46,** 1000 (1913).

[80] Siedel, H. Fischer: *Z. physiol. Chem.*, **214,** 145 (1933).

[81] Synthesis: H. Fischer, Hartmann: *Ibid.*, **226,** 116 (1934).

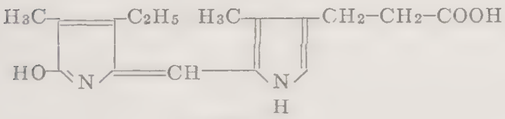
The constitution of bilirubic acid[82] was deduced from the following experimental findings. On heating with potassium methoxide it undergoes fission to trimethylpyrrylpropionic acid, whereas with sodium methoxide, xanthobilirubic acid and eventually the same trimethylpyrrylpropionic acid were obtained (at this time isoxanthobilirubic acid had not yet been found). When both formulas[83] had been established by analytical methods, the constitutions were completely confirmed by synthesis. Replacement of the labile bromine atom of 4,3′,5′-trimethyl-3-ethyl-5-brompyrrolmethene-4′-propionic acid (hydrobromide) (II)[84]:



(II)

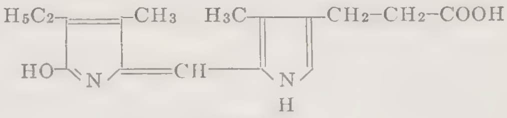
by the action of potassium acetate gave xanthobilirubic acid[85] which was reduced by sodium amalgam to synthetic bilirubic acid.[86] A mesoporphyrin[87] may be synthesized from bilirubic acid and from mesobilirubinogen in agreement with the dipyrrylmethane structure assumed for them.

Fusion of mesobilirubin[88] with resorcinol yields neoxanthobilirubic acid (I) and an isomeric isoneoxanthobilirubic acid (II)[89]:



(m.p. 245°)

(I)



(m.p. 247°)

(II)

catalytic reduction converting them into neobilirubic (III) and isoneobilirubic acids (IV):

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[82] H. Fischer, Bartholomäus: *Ibid.*, **83**, 50 (1913); **87**, 255 (1913); H. Fischer, Röse: *Ibid.*, **89**, 255 (1913); **91**, 184 (1914); *Ber.*, **47**, 791 (1914); H. Fischer, Eismayer: *Ber.*, **47**, 2019 (1914); Piloty, Stock, Dormann: *Ann.*, **406**, 342 (1914).

[83] H. Fischer, Hess: *Z. physiol. Chem.*, **194**, 193 (1931).

[84] H. Fischer, Berg: *Ann.*, **482**, 189 (1930); cf. H. Fischer, Fröwis: *Z. physiol. Chem.*, **195**, 49 (1931); H. Fischer, Yoshioka, Hartmann: *Ibid.*, **212**, 146 (1932).

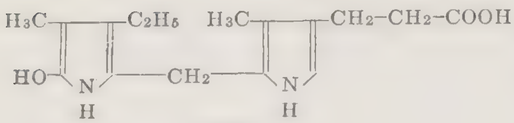
[85] H. Fischer, Adler: *Ibid.*, **197**, 237 (1931); synthesis of compounds of the type of xanthobilirubic acid: H. Fischer, Loy: *Z. physiol. Chem.*, **128**, 59 (1923); earlier work: Küster: *Ibid.*, **94**, 136 (1915); *Arch. Pharm.*, **253**, 457 (1915); *Z. physiol. Chem.*, **99**, 86 (1917); **121**, 80, 94 (1922); Küster, Herrmann: *Ibid.*, **121**, 110 (1922); Küster, Maag: *Ber.*, **56**, 55 (1923); H. Fischer, Barrenscheen: *Z. physiol. Chem.*, **115**, 94 (1921); H. Fischer, Niemann: *Ibid.*, **127**, 317 (1923); H. Fischer, Schubert: *Ber.*, **56**, 2379 (1923); H. Fischer, Müller. *Z. physiol. Chem.*, **132**, 72 (1924); Müller: *Ibid.*, **135**, 108 (1924); H. Fischer, Niemann: *Ibid.*, **137**, 293 (1924); **146**, 196 (1925).

[86] H. Fischer, Röse: *Ber.*, **46**, 441 (1913); H. Fischer, Adler: *Z. physiol. Chem.*, **197**, 237 (1931).

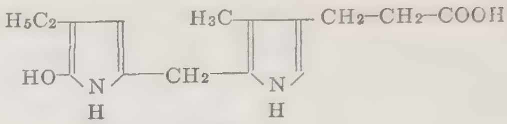
[87] H. Fischer, Lindner: *Ibid.*, **161**, 1 (1926).

[88] H. Fischer, Hess: *Ibid.*, **194**, 193 (1931); Siedel, H. Fischer: *Ibid.*, **214**, 145 (1933); this fission consists in introducing the compound under investigation into boiling resorcinol.

[89] Siedel, H. Fischer: *Ibid.*, **214**, 145 (1933).

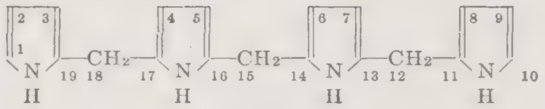

H3C
C2H5
H3C
CH2–CH2–COOH
HO
N
H
CH2
N
H

(m.p. 179°)
(III)


H5C2
H3C
CH2–CH2–COOH
HO
N
H
CH2
N
H

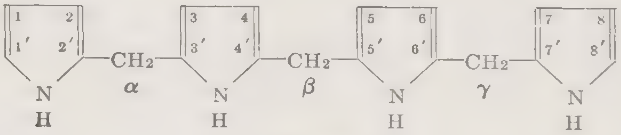
(m.p. 194°)
(IV)

Synthesis of mesobilirubin[90] has been effected, showing that 17 carbon atoms are accounted for by bilirubic acid and 16 carbon atoms by neoxanthobilirubic acid or its iso-derivatives (see later the synthesis of mesobilirubin). Thus 33 carbon atoms as demanded by the formula have been recognized by degradative and synthetic means. Degradation of mesobilirubin and examination of its fission products therefore has led to the deduction that bilirubin and its associated compounds are derived from a parent compound of type (V):

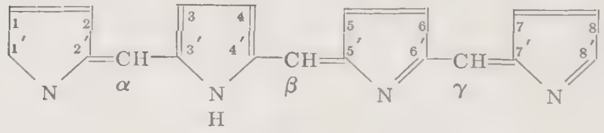

2
3
1
N
H
19
18
CH2
17
4
5
N
H
16
15
CH2
14
6
7
N
H
13
12
CH2
11
8
9
N
H
10

(V)

More recently the numbering of the atoms in bilane,[91] as this compound is termed, has been modified as is shown below, bilin, its dehydrogenation product, being similarly numbered:


1
2
1′
2′
N
H
CH2
α
3
4
3′
4′
N
H
CH2
β
5
6
5′
6′
N
H
CH2
γ
7
8
7′
8′
N
H

Bilane


1
2
1′
2′
N
=CH–
α
3
4
3′
4′
N
H
–CH=
β
5
6
5′
6′
N
–CH=
γ
7
8
7′
8′
N

Bilin

Mesobilirubins, as has already been mentioned, have also been obtained synthetically, *e.g.*, symmetrical mesobilirubins may be obtained from brompyrromethane bromides[92] (the isomerides differ according to the orientation of the side chains); a total of 52 mesobilirubins[93] and as many bilirubins are theoretically possible:

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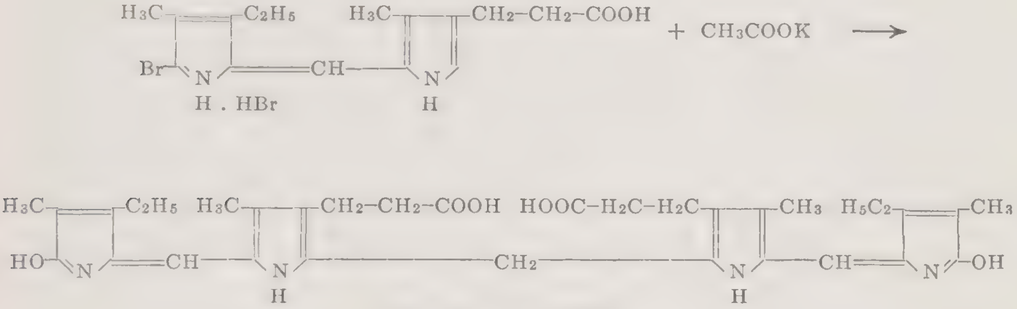
[90] H. Fischer, Adler: *Ibid.*, **200**, 212 (1931); Siedel, H. Fischer: *Ibid.*, **214**, 145 (1933); H. Fischer, Hartmann: *Ibid.*, **226**, 116 (1934); H. Fischer, Siedel: *Ibid.*, **231**, 167 (1935); H. Fischer, Höfelmann: *Ibid.*, **251**, 187 (1938).

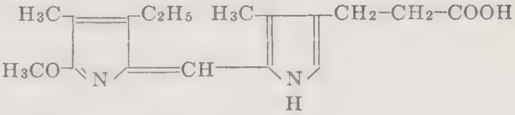
[91] H. Fischer, Haberland: *Z. physiol. Chem.*, **232**, 236 (1935).

[92] Siedel, H. Fischer: *Ibid.*, **214**, 145 (1933), where questions of nomenclature are considered; a mesobilirubin synthesis had been carried out earlier, *e.g.*, H. Fischer, Adler: *Ibid.*, **200**, 227 (1931) although the first so-called K-mesobilirubin was a mixture [H. Fischer, Hess: *Ibid.*, **194**, 193 (1931)]; further synthetic experiments: H. Fischer, Aschenbrenner: *Ibid.*, **229**, 71 (1934).



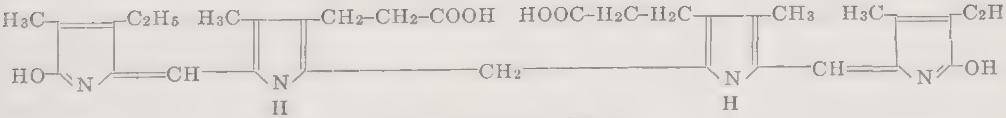
Mesobilirubin XIII α

Other methods of synthesizing mesobilirubins include the action of sodium methoxide on the methyl ether of neoxanthobilirubic acid (I) or of formaldehyde on neoxanthobilirubic acid itself:



(I)

Mesobilirubin isolated from bile pigment has thus been shown to have the formula:



Mesobilirubin IX α

It has been synthesized[94] from hydroxymethylneoxanthobilirubic acid (II) and isoneoxanthobilirubic acid:

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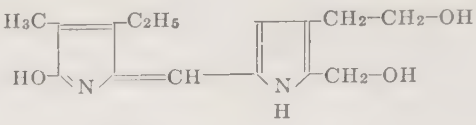
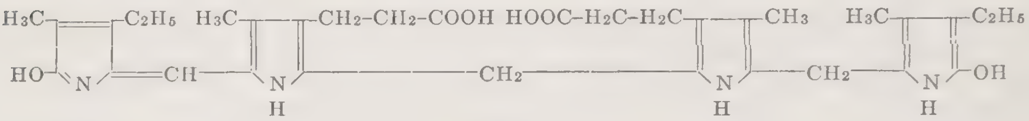
[93] 15 hemins (for the relation between hemin and bilirubin, see later) are possible [H. Fischer, Stangler: *Ann.*, **459**, 62 (1927)] from most of which four bilirubins or four mesobilirubins are obtainable by fission. When the hemin possesses one plane of symmetry, three bilirubin isomerides are to be expected, and two will arise if the hemin possesses two planes of symmetry; XIII signifies its relation to the hemin of that number, the Greek suffix indicating which methine bridge has been opened.

[94] Siedel: *Z. physiol. Chem.*, **245**, 257 (1937).

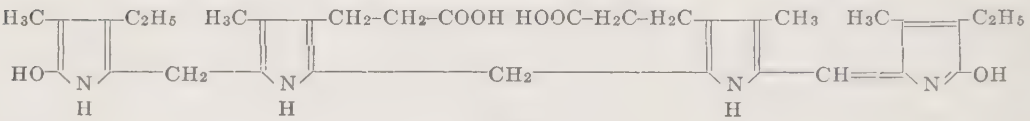

H₃C
C₂H₅
HO
N
CH
CH₂–CH₂–OH
CH₂–OH
N
H

(II)

Dihydromesobilirubin,[95] containing two more atoms of hydrogen, must have one of the two formulas (III) or (IV):

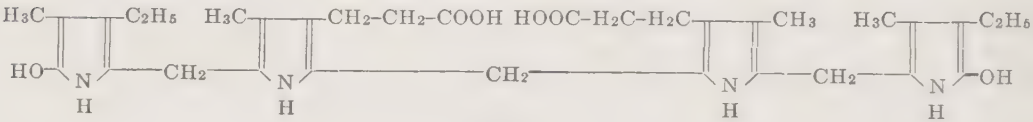

H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
H₃C
C₂H₅
HO
N
CH
N
H
CH₂
N
H
CH₂
N
H
OH

(III)


H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
H₃C
C₂H₅
HO
N
H
CH₂
N
H
CH₂
N
H
CH
N
OH

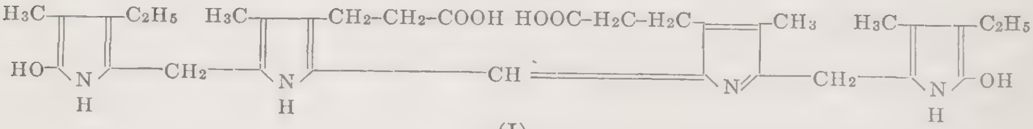
(IV)

for the still further hydrogenated mesobilirubinogen (urobilirubinogen), the following constitution (V) must be assumed:


H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
H₃C
C₂H₅
HO
N
H
CH₂
N
H
CH₂
N
H
CH₂
N
H
OH

(V)

Urobilin,[96] which is produced by atmospheric oxidation of mesobilirubinogen, is given the formula (I):


H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
H₃C
C₂H₅
HO
N
H
CH₂
N
H
CH
N
CH₂
N
H
OH

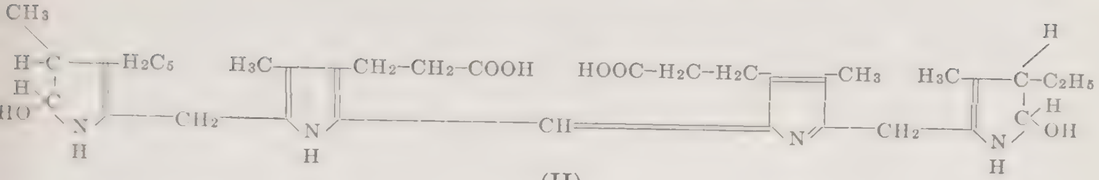
(I)

and stercobilin, present in faeces, has the closely related hydrated structure (II):

---

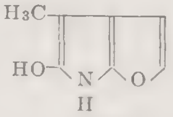
[95] H. Fischer, Baumgartner: *Ibid.*, **216**, 260 (1933).

[96] Literature, see H. Fischer and H. Orth: "Die Chemie des Pyrrols," Vol. **II**, 1. p. 675; synthesis of urobilin: Siedel: *Angew. Chem.*, **49**, 554 (1936); Siedel, Meier: *Z. physiol. Chem.*, **242**, 101 (1936); H. Fischer, Libowitzky: *Ibid.*, **258**, 255 (1939).

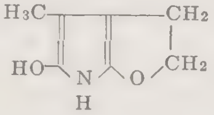

CH3
H-C
H2C5
H
C
HO
N
H
H3C
CH2
CH2-CH2-COOH
N
H
CH
HOOC-H2C-H2C
CH3
N
CH2
H3C
H
C-C2H5
H
C
OH
N
H

(II)

Returning now to the constitution of bilirubin, the natural pigment, it may be recalled that its catalytic reduction is undoubtedly initially concerned with the saturation of double bonds which are not essential to the chromophoric system. The assumption of two vinyl groups in place of ethyl groups would be completely justified, having regard to the close relation to hemin were it not that oxidation of bilirubin with nitrite gives the compound (III), the constitution of which was placed beyond doubt by the formation of (IV) by catalytic reduction:

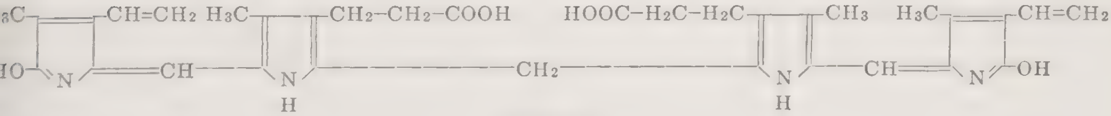


(III)


H3C
CH2
CH2
HO
N
H
O

(IV)

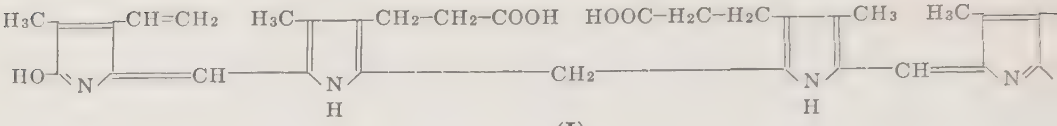
Moreover, the ready reduction of bilirubin by sodium amalgam is remarkable when it is remembered that hemin, which certainly contains vinyl groups, is unaffected by this reagent. It is, however, certain that the conversion of hemin into bilirubin includes the oxidative elimination of the α-methine bridge and the appearance of two hydroxyl groups on nuclei I and IV. Thus the same orientation of β-substituents in bilirubin as is found in hemin was early regarded as probable and is today confirmed by the discovery of the series of iso-acids among the degradation products of bilirubin derivatives. Thus the following structure has been proposed for bilirubin:


CH=CH2
H3C
CH2-CH2-COOH
HOOC-H2C-H2C
CH3
H3C
CH=CH2
N
CH
N
H
CH2
N
H
CH
N
OH

Bilirubin

It remains problematical whether a furane ring[97,100] is linked by ring formation between the α-hydroxy and β-vinyl group of pyrrole ring IV. In the latter case the natural pigment would have the formula (I):

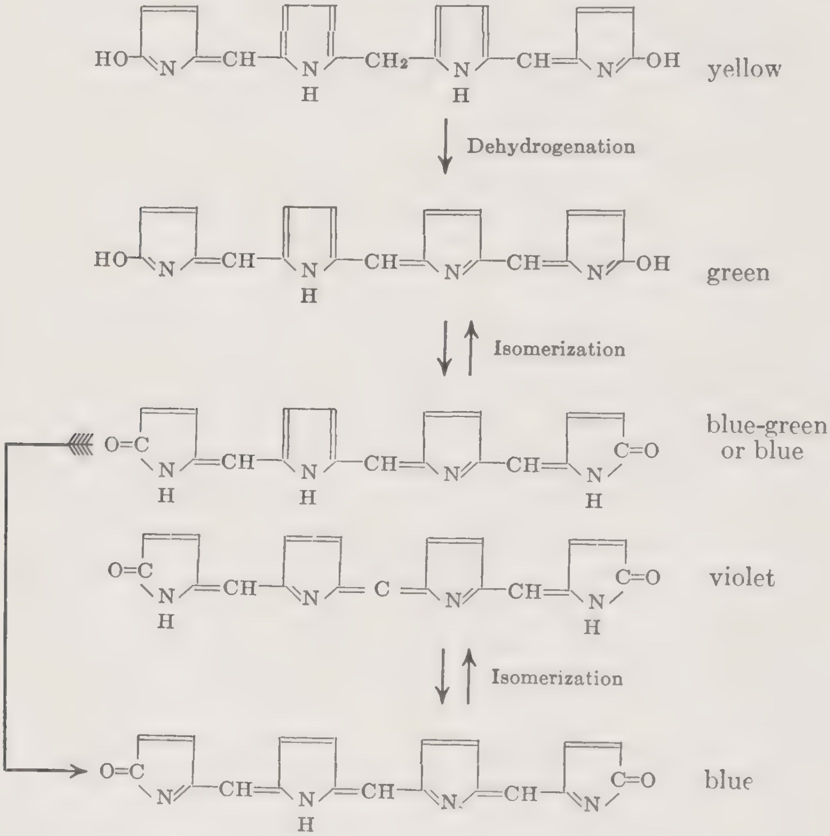
[97] Cf. also H. Fischer, Reinecke: *Z. physiol. Chem.*, **258**, 9 (1939).



(I)

Experiments to synthesize bilirubin [98] have not yet reached final success.

H. Fischer and his co-workers have devoted painstaking research [99] to the mechanism of the Gmelin reaction mentioned above, which is in reality an indication of a quinonoid state. The reaction consists in dehydrogenating a system of four pyrrole rings giving rise to different stages of oxidation in the following manner [100]:



Further reaction results in oxidative fission to maleimides.

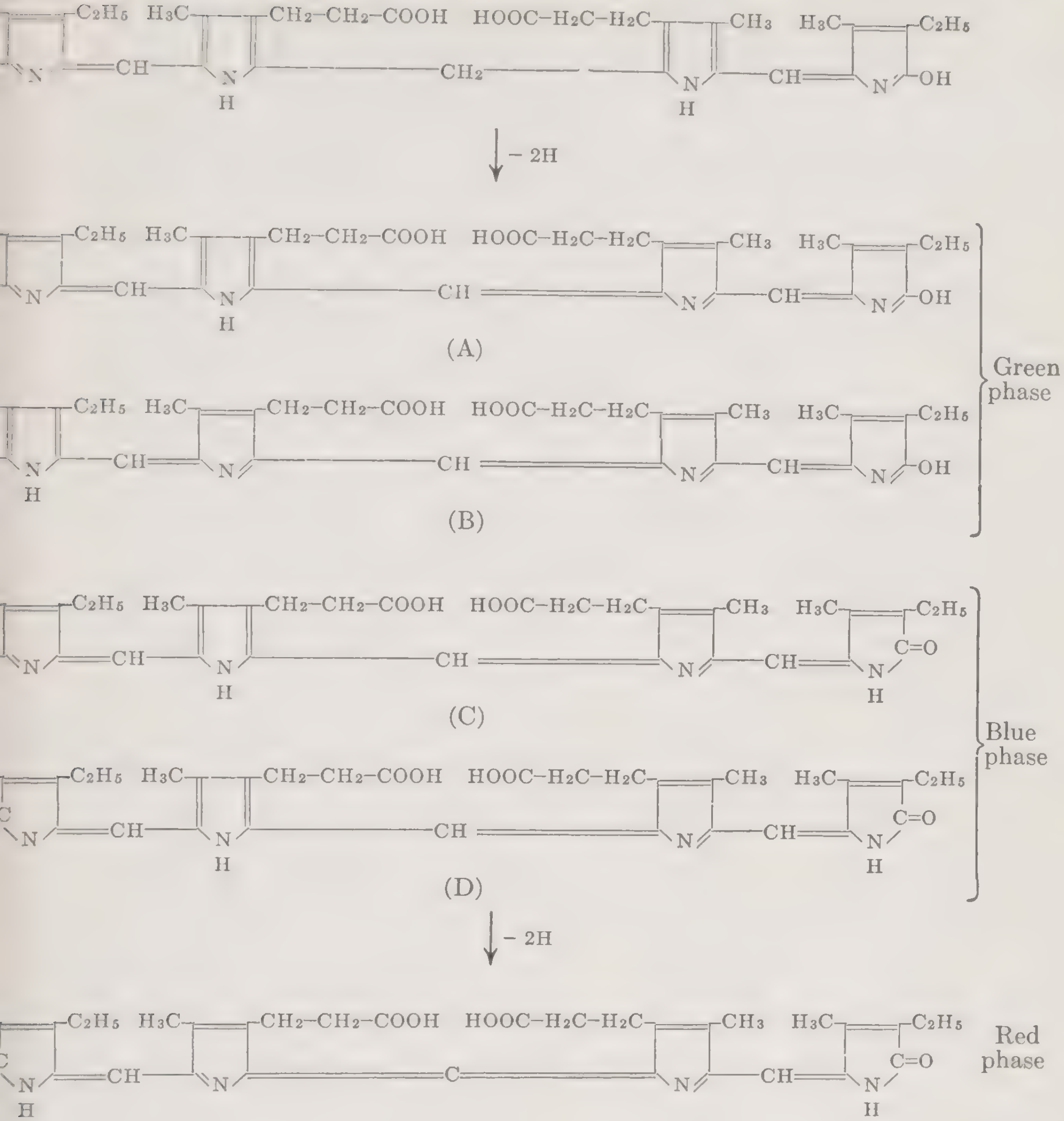
At one time the color change of, for example, mesobilirubin IXα from green through blue to red was interpreted as proceeding through the following stages. A, B, C and D are isomerides.

---

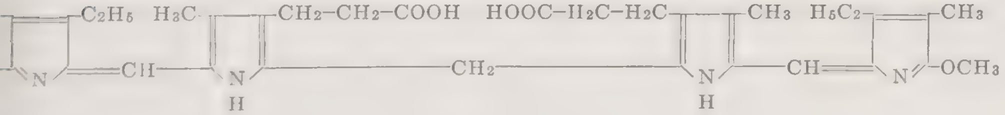
[98] H. Fischer, Adler: *Ibid.*, 200, 209 (1931); 210, 139 (1932).

[99] H. Fischer, Adler: *Ibid.*, 206, 187 (1932); Siedel, H. Fischer: *Ibid.*, 214, 145 (1933); H. Fischer, Fries: *Ibid.*, 231, 231 (1935); H. Fischer, Aschenbrenner: *Ibid.*, 245, 107 (1937).

[100] H. Fischer, Haberland: *Ibid.*, 232, 236 (1935).

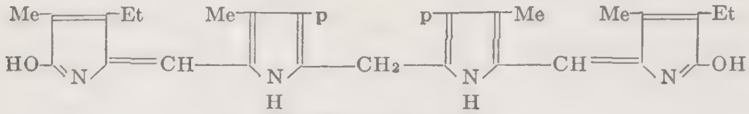


There is available very strong evidence that this postulated mechanism is the correct one in the behavior of a mesobilirubin dimethyl ether of the constitution:

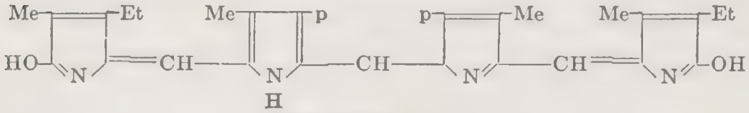


This is obtained by the action of formaldehyde on the methyl ether of neoxanthobilirubic acid; it is clearly unable to assume a quinonoid state, and indeed the Gmelin reaction does not proceed beyond the green phase.

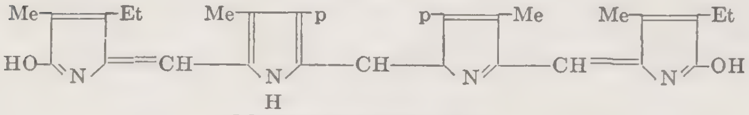
More careful examination of the reaction has shown that the compounds responsible for the color vary somewhat according to the conditions. Using bromine in methanol as the oxidizing agent, some at least of the highly colored intermediates are methoxyl compounds. The following show intermediates isolated by stopping the reaction when still incomplete and chromatographing the product[100a]:


Me
Et
HO
N
CH
Me
p
N
H
CH₂
p
Me
N
H
CH
Me
Et
N
OH

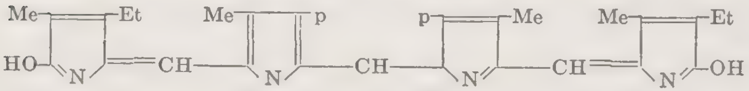
Mesobilirubin XIII


Me
Et
HO
N
CH
Me
p
N
H
CH
p
Me
N
CH
Me
Et
N
OH

Glaucobilin XIII

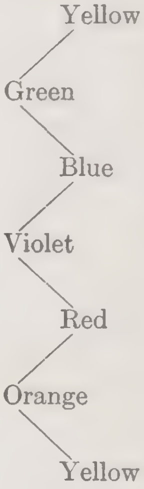

Me
Et
HO
N
CH
Me
p
N
H
CH
p
Me
N
CH
Me
Et
N
OH

Mesobilipurpurin XIII

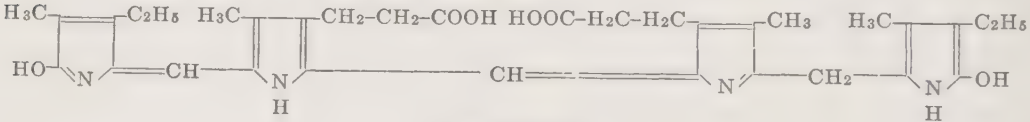

Me
Et
HO
N
CH
Me
p
N
CH
p
Me
N
CH
Me
Et
N
OH

Hesocholetelin XIII

(p = $\cdot CH_2CH_2COOH$ or $\cdot CH_2CH_2CO_2CH_3$)

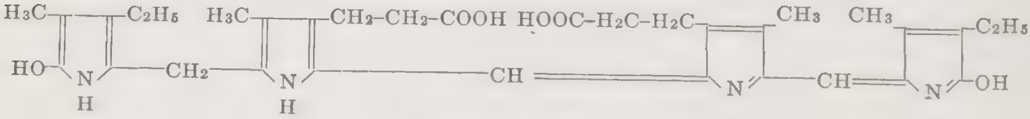

Yellow
Green
Blue
Violet
Red
Orange
Yellow

Mesobiliviolin,[101] another degradation product of mesobilirubinogen, has the structure:


H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
H₃C
C₂H₅
HO
N
CH
N
H
CH
N
CH₂
N
H
OH

Mesobiliviolin

and mesobilirhodin[102] is regarded as possessing the constitution:

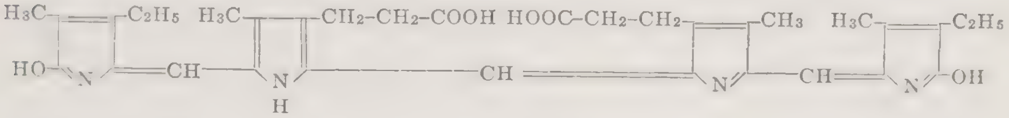

H₃C
C₂H₅
H₃C
CH₂–CH₂–COOH
HOOC–H₂C–H₂C
CH₃
CH₃
C₂H₅
HO
N
H
CH₂
N
H
CH
N
CH
N
OH

Mesobilirhodin

Although this work is limited to natural pigments, there are two derivatives, ferrobilin and glaucobilin, which merit consideration. Ferrobilin is a chloroiron salt obtained by the action of ferric chloride in acetic acid

[100a] Siedel, Fröwis, *Z. physiol. Chem.*, **267**, 37 (1940).
[101] Siedel: *Ibid.*, **237**, 33 (1935).
[102] Siedel: *Ibid.*, **237**, 8 (1935).

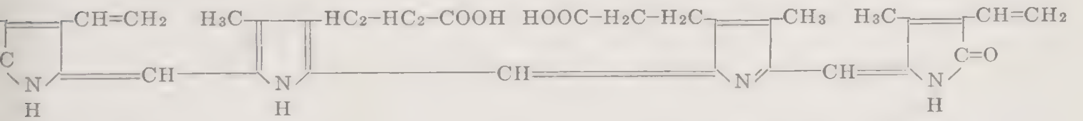
on mesobilirubin; its color is blue-violet. Glaucobilin is the iron-free pigment:



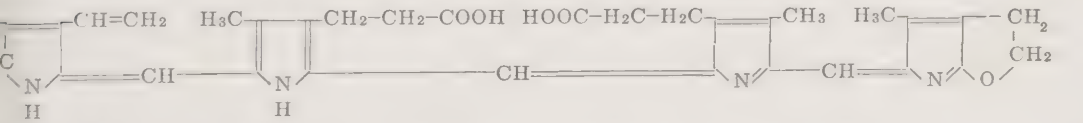
a dehydromesobilirubin the constitution of which is removed beyond doubt by its synthesis.[103] It has not been observed in nature, but pigments [104] are known which may readily be converted into glaucobilin; and it has been suggested that glaucobilin is responsible for the first blue phase of a traumatic hematom. No proof of this exists, although glaucobilin is rapidly degraded after subcutaneous injection.

A number of investigations into the degradation of blood to bile pigment deserve mention here as they throw some light on the mechanism. The so-called green hemins of Warburg and Negelein [105] are probable intermediates as they may be converted into a dehydrobilirubic ester,[106] which is also obtainable from bilirubin. Probably more significant, however, is the conversion [107] of coprohemin ester I, in the presence of ascorbic acid, into coproglaucobilin ester I, as this marks the first occasion on which a homogeneous crystalline bile pigment was obtained from a hemin by a route which may conceivably play a part in the metabolism of the living cell. The production of bilirubin from hemoglobin can also be effected biochemically in the presence of liver tissue.

Uteroverdin,[108] the coloring matter of the green parts of the placenta of the dog, is a dehydrobilirubin, probably having one of the following constitutions:



or



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[103] Siedel: *Ibid.*, **237,** 12 (1935).

[104] Lemberg, Bader: *Naturwiss.*, 21, 206 (1933); Lemberg: *Ann.*, **505,** 151 (1933); cf. also Lemberg: *Biochem. J.*, **29,** 1326 (1935).

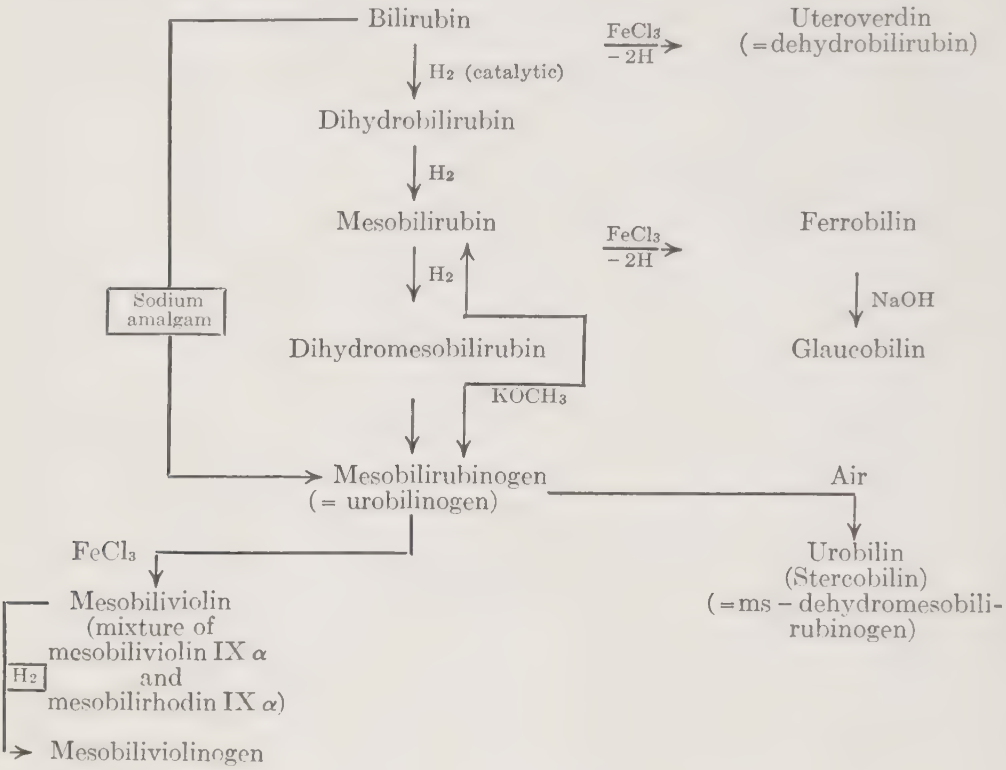
[105] Warburg, Negelein: *Ber.*, **63,** 1816 (1930).

[106] Lemberg: *Biochem. J.*, **29,** 1322 (1935).

[107] H. Fischer, Libowitzky: *Z. physiol. Chem.*, **251,** 198 (1938); on the pentdyopent reaction, cf. H. Fischer, Müller: *Ibid.*, **246,** 43 (1937); H. Fischer, Reinecke, Lichtenwald: *Ibid.*, **257,** 190 (1939); degradation of hemoglobin: Lemberg: *Chem. Zentr.*, **1939,** II, 2333; preliminary account of other pigments similar to bilirubin: Siedel, Möller. *Z. physiol. Chem.*, **259,** 122 (1939); Meldolesi, Siedel, Möller: *Ibid.*, **259,** 137 (1939); Waldenström, Vahlquist: **260,** 189 (1939).

[108] Lemberg, Barcroft: *Proc. Roy. Soc. London (B)*, **110,** 362 (1932); Lemberg: *Ann.*, **499,** 25 (1933); *Biochem. J.*, **28,** 978 (1934); H. Fischer, Hess, Baumgartner: *Z. physiol. Chem.*, **206,** 202 (1932); Siedel, H. Fischer: *Ibid.*, **214,** 155 (1933); H. Fischer, Haberland: *Ibid.*, **232,** 243 (1935).

The relations between the various oxidation and reduction products of bilirubin [109] are summarized in the following table:


Bilirubin
H₂ (catalytic)
FeCl₃
− 2H
Uteroverdin
(=dehydrobilirubin)
Dihydrobilirubin
H₂
Mesobilirubin
FeCl₃
− 2H
Ferrobilin
NaOH
Glaucobilin
H₂
Sodium amalgam
Dihydromesobilirubin
KOCH₃
Mesobilirubinogen
(= urobilinogen)
Air
Urobilin
(Stercobilin)
(=ms − dehydromesobilirubinogen)
FeCl₃
Mesobiliviolin
(mixture of
mesobiliviolin IX α
and
mesobilirhodin IX α)
H₂
Mesobiliviolinogen

Oöcyan [110] is a blue or blue-green pigment found in the shells of the eggs of many wild birds. It is closely related to bilirubin, as the recent empirical formula of its diester, $C_{37}H_{40}O_8N_4$, would indicate.

Phycobilins.[111] The chromatophors of red algae contain, in addition to the normal pigments of green plants, red and blue water-soluble pigments, phycoerythrin and phycocyan, associated with protein. Their role would seem to be that of sensitizers, enabling the algae to inhabit regions of the sea where the absorption of light by chlorophyll is no longer possible. The terms *phycoerythrobilin* and *phycocyanobilin* have recently been proposed. Glaucobilin may be obtained from each of these pigments, but no final agreement on their constitutional formulas has yet been reached.

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[109] H. Fischer and H. Orth: "Die Chemie des Pyrrols," Vol. **II,** 1, p. 623.

[110] H. Fischer, Kögl: *Z. physiol. Chem.*, **131,** 241 (1923); H. Fischer, Lindner: *Ibid.*, **145,** 216 (1925); Lemberg: *Ann.*, **488,** 74 (1931), **499,** 32 (1932); The wings of the cabbage butterfly contain a pigment which resembles oöcyan: Wieland, Kotzschmar: *Ann.*, **530,** 152 (1937) and particularly p. 155.

[111] Kylin: *Z. physiol. Chem.*, **69,** 169 (1910); **76,** 396 (1912); Lemberg: *Ann.*, **461,** 56 (1928); **477,** 195 (1930); **505,** 172 (1933); cf. also Kitasato: *Acta phytochimica*, **2,** 75 (1925); Siedel: *Z. physiol. Chem.*, **237,** 21 (1935); Eriksson-Quensel: *Biochem. J.*, **32,** 585 (1938). Description of a mussel pigment, rufescin, which appears to be closely related to the bile pigments: Dhéré, Baumler: *Arch. intern. physiol.*, **32,** 55 (1930); cf. Schulz, Becker: *Biochem. Z.*, **236,** 99 (1931).

Pterobilin is a blue pigment present in many Pieris species though it is quite different from the pterines. Eight mg. of pigment were obtained from the wings (125 g.) of 6000 insects (Catopsilia rurina). Pterobilin is a dicarboxylic acid and its dimethyl ester, $C_{35}H_{38}O_6N_4$, m.p. 234°, still contains two hydroxyl groups and one imino group. The formation of metal complexes, the Gmelin reaction, and other properties indicate that pterobilin belongs to the bile pigment series; it appears to contain similar substituents, though their arrangement must be different.[111a]

Chlorophyll.[112] Many workers since the time of Berzelius[113] have been attracted by the problem of the constitution of chlorophyll, as the green pigment of the leaf was termed by Pelletier and Caventou. Stokes[114] discovered spectroscopically that chlorophyll is a mixture and used a process for separating the compounds—partitioning them between alcohol and carbon disulfide—which was also employed by Sorby[115] and later rediscovered and elaborated by Kraus.[116] A relationship between the pigments of blood and leaves was postulated as long ago as 1851 by Verdeil,[117] and although this hypothesis was pursued by Hoppe-Seyler,[118] Schunck[119] and Marchlewski,[120] and is now known to be correct, the original observations on which it was founded have been discredited by the advancing knowledge of the leaf pigments.

Attempts to isolate chlorophyll were abandoned for some time after the work of Hoppe-Seyler[121] and Gautier[122] in view of its sensitiveness, its ready solubility and the difficulty of separating it from colorless and colored concomitants.

Chlorophyll occurs in the plant only in certain plasmic bodies, the chloroplasts (chlorophyll granules). The same chlorophylls are found in all foliage plant life from algae to flowering plants, where they are most abundant in the leaves and bark; they occur with an approximately constant proportion of carotenes and xanthophylls.

The isolation of chlorophyll from nettle leaves was achieved in 1911 by Willstätter[123] by a systematic extension of the partition methods supported by repeated colorimetric determinations of the purity of his solutions. In this manner the leaf pigment may be isolated from plants with the same ease as serves for the isolation of a sugar or alkaloid. All plants yet examined contain the same chlorophyll consisting of two compounds:

[111a] Wieland, Tartter, *Ann.*, **545**, 197 (1940).

[112] Earlier literature: R. Willstätter and A. Stoll: "Untersuchungen über Chlorophyll," Berlin, 1913; R. Willstätter and A. Stoll: "Untersuchungen über die Assimilation der Kohlensäure," Berlin, 1918; Willstätter: *Ber.*, **47**, 2831 (1914) (lecture review); more recent reviews: C. C. Steele: *Chem. Rev.*, **20**, 1 (1937); H. Fischer: *Ibid.*, **20**, 41 (1937); R. P. Linstead: *Ann. Rep.*, **34**, 375 (1937); A. Stoll and E. Wiedemann: "Fortschritte der Chemie der organischen Naturstoffe," Berlin, 1938, **I**, 159 (complete literature 1906-1938).

[113] Berzelius: *Ann.*, **27**, 296 (1838).

[114] Stokes: *Proc. Roy. Soc. London*, **13**, 144 (1864); cf. Tswett: "Chromophylls in the Plant and Animal Kingdom," Warsaw, 1910.

[115] Sorby: *Proc. Roy. Soc. London*, **15**, 433 (1867); **21**, 442 (1873).

[116] Kraus: "Zur Kenntnis der Chlorophyllfarbstoffe und ihrer Verwandten," Stuttgart, 1872.

[117] Verdeil: *Compt. rend.*, **33**, 689 (1851).

[118] Hoppe-Seyler: *Z. physiol. Chem.*, **4**, 193 (1880).

[119] Schunck: *Proc. Roy. Soc. London*, **39**, 348 (1885); **44**, 448 (1888).

[120] Marchlewski: "Chemie der Chlorophylle," Braunschweig, 1909.

[121] Hoppe-Seyler: *Z. physiol. Chem.*, **3**, 339 (1879).

[122] Gautier: *Compt. rend.*, **89**, 861 (1879).

[123] Willstätter, Hug: *Ann.*, **380**, 177 (1911); improved methods: R. Willstätter and A. Stoll: "Untersuchungen über Chlorophyll," Chaps. III and IV.

Chlorophyll *a*: $C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b*: $C_{55}H_{70}O_6N_4Mg$;

but nettles are particularly suitable for the preparation of chlorophyll as they contain only a little chlorophyllase (see later). More recently the separation of the two compounds has been achieved by Winterstein and Stein[124] with the assistance of chromatographic analysis. The two compounds show the following absorption spectra (ether):

Chlorophyll *a*: 663-623-607-577-534-507-494-432 $m\mu$
Chlorophyll *b*: 644-614-594-567-542-503-456-428 $m\mu$

It will be noted that the two chlorophylls[125] are complementary with respect to their absorption spectra, so that the plant is enabled to utilize the energy of the sun throughout the whole spectral range. The ratio of *a* to *b* in the plant is about 1:2.9. Both pigments are microcrystalline, the *a* compound being obtained in spear-shaped leaflets on slow evaporation.

The chlorophylls undergo fission into carboxylic acids and an alcohol in the presence of an enzyme, chlorophyllase, which accompanies the pigments in the plant. Thus the formula of chlorophyll *a* may be partially resolved[126]:

$$C_{55}H_{72}O_5N_4Mg + H_2O = \underset{(\textit{Chlorophyllide } a)}{C_{34}H_{33}O_3N_4MgCOOH} + \underset{(\textit{Phytol})}{C_{20}H_{39}OH}$$

Chlorophyllide *a* has been observed by Borodin[127] but "crystalline chlorophyll" usually denotes the ethyl ester produced from the natural phytyl ester. Thus, if chlorophyll is allowed to stand in alcoholic solution, no chlorophyllide is formed but the ester of the alcohol used as solvent is obtained, *e.g.*, ethyl chlorophyllide *a*,[128] $C_{34}H_{33}O_3N_4Mg(CO_2C_2H_5)$.

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[124] Winterstein, Stein: *Z. physiol. Chem.*, **220**, 263 (1933); cf. Zscheile, Jr., *J. physic. Chem.*, **38**, 95 (1934); *Botan. Gaz.*, **95**, 529 (1934); Winterstein, Schön: *Z. physiol. Chem.*, **230**, 139 (1934); Bakker: *Chem. Zentr.*, **1935**, *I*, 2189; A. Stoll and E. Wiedemann: "Fortschritte der organischen Naturstoffe," p. 185; for chlorophyll formulas, see Stoll, Wiedemann: *Helv. Chim. Acta*, **16**, 739 (1933).

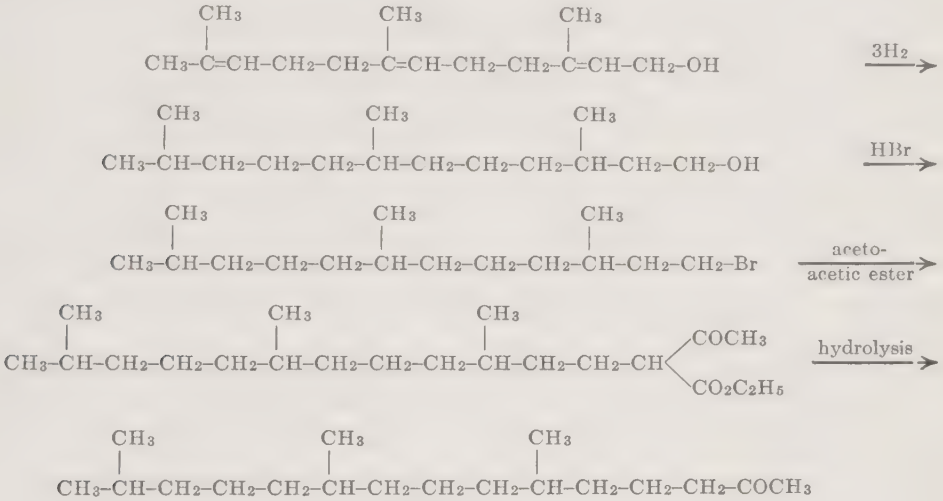
[125] Conant, Dietz [*Nature*, **131**, 131 (1933)] believe that chlorophylls *a* and *b* both exist in two forms: cf. in this connection Zscheile, Jr., *Botan. Gaz.*, **95**, 529 (1934). Constitution and absorption of chlorophyll derivatives: Pruckner: *Z. physik. Chem.*, **187A**, 257 (1940).

[126] Willstätter, Benz: *Ann.*, **358**, 267 (1908), where earlier literature is cited. Synthesis of chlorophyll from chlorophyllide and phytol: Willstätter, Stoll: *Ann.*, **380**, 148 (1911).

[127] Borodin: *Botan. Ztg.*, **40**, 608 (1882).

[128] H. Fischer, Spielberger: *Ann.*, **515**, 131 (1935).

The elucidation of the constitution of phytol was begun by Willstätter[129] and successfully concluded by F. G. Fischer.[130] Ozonolysis of phytol yields glycol aldehyde and a ketone, $C_{18}H_{36}O$, which was shown to be 2,6,10-trimethylpentadecan-14-one (I) and was synthesized as follows from farnesol:



$$CH_3-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-OH \xrightarrow{3H_2}$$

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-OH \xrightarrow{HBr}$$

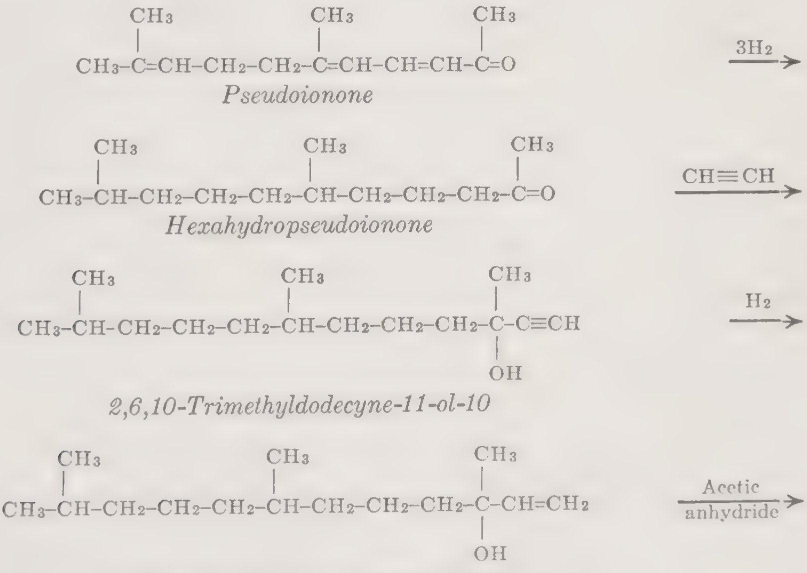
$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-Br \xrightarrow[\text{acetic ester}]{\text{aceto-}}$$

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH\left\langle\begin{matrix}COCH_3\\ CO_2C_2H_5\end{matrix}\right. \xrightarrow{\text{hydrolysis}}$$

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-COCH_3$$

(I)

The synthesis of phytol itself was then carried out from pseudoionone as starting material:



$$CH_3-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH=CH-\overset{CH_3}{\overset{|}{C}}=O \xrightarrow{3H_2}$$

Pseudoionone

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=O \xrightarrow{CH\equiv CH}$$

Hexahydropseudoionone

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\underset{\underset{OH}{|}}{\overset{CH_3}{\overset{|}{C}}}-C\equiv CH \xrightarrow{H_2}$$

2,6,10-Trimethyldodecyne-11-ol-10

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\underset{\underset{OH}{|}}{\overset{CH_3}{\overset{|}{C}}}-CH=CH_2 \xrightarrow[\text{anhydride}]{\text{Acetic}}$$

2,6-Dimethyl-10-vinyl-undecan-10-ol

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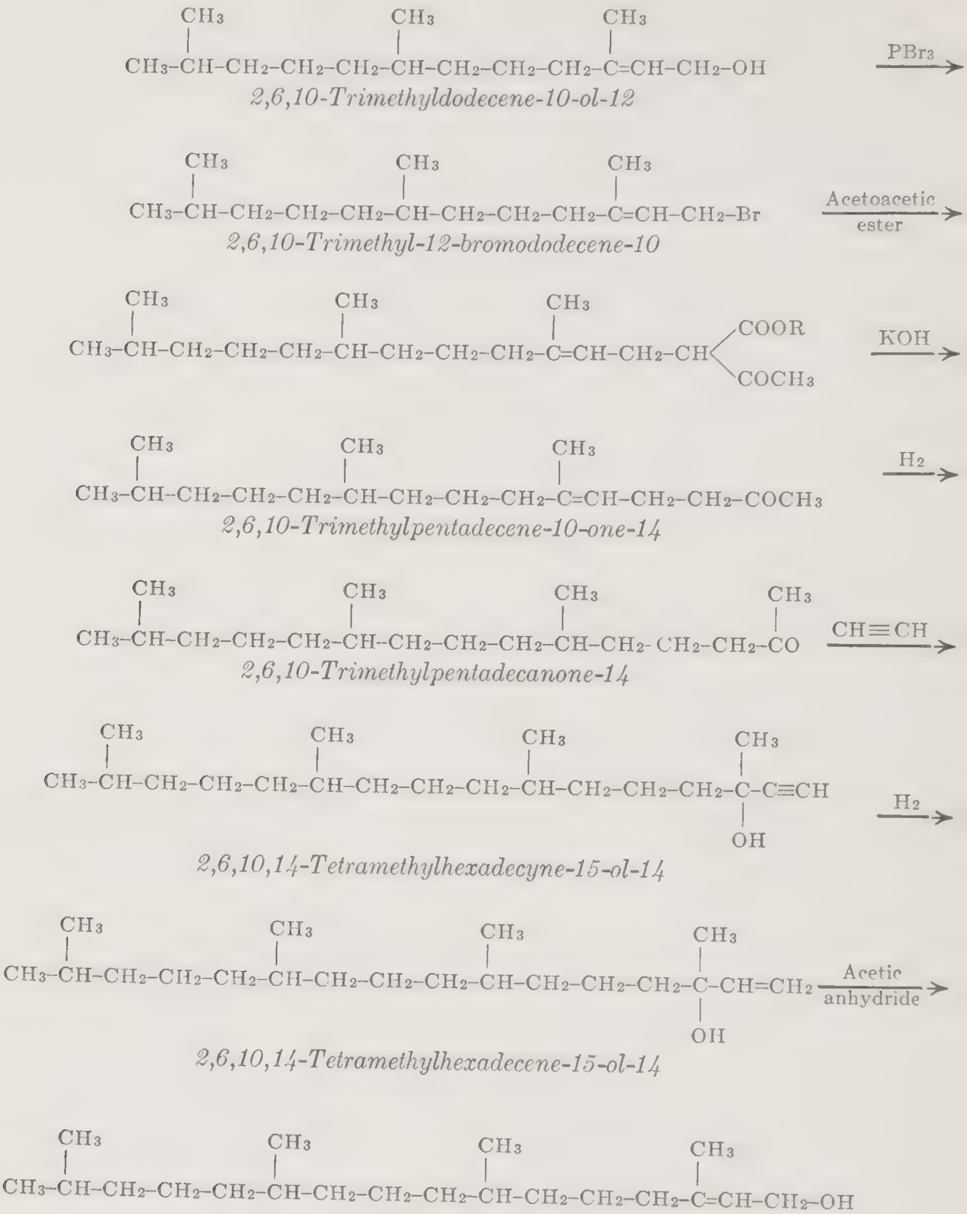
[129] Willstätter, Hocheder: *Ann.*, **354**, 205 (1907); Willstätter, Mayer, Hüni: *Ann.*, **378**, 73 (1911); Willstätter, Schuppli, Mayer: *Ann.*, **418**, 121 (1919).

[130] F. G. Fischer: *Ann.*, **464**, 69 (1928); F. G. Fischer, Löwenberg: *Ann.*, **475**, 183 (1929); cf. also Karrer, Ringier: *Helv. Chim. Acta*, **22**, 610 (1939).



$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-OH \xrightarrow{PBr_3}$$

2,6,10-Trimethyldodecene-10-ol-12

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-Br \xrightarrow[\text{ester}]{\text{Acetoacetic}}$$

2,6,10-Trimethyl-12-bromododecene-10

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH\begin{matrix} \diagup COOR \\ \diagdown COCH_3 \end{matrix} \xrightarrow{KOH}$$

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-COCH_3 \xrightarrow{H_2}$$

2,6,10-Trimethylpentadecene-10-one-14

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}O} \xrightarrow{CH\equiv CH}$$

2,6,10-Trimethylpentadecanone-14

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\underset{OH}{\underset{|}{\overset{CH_3}{\overset{|}{C}}}}-C\equiv CH \xrightarrow{H_2}$$

2,6,10,14-Tetramethylhexadecyne-15-ol-14

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\underset{OH}{\underset{|}{\overset{CH_3}{\overset{|}{C}}}}-CH=CH_2 \xrightarrow[\text{anhydride}]{\text{Acetic}}$$

2,6,10,14-Tetramethylhexadecene-15-ol-14

$$CH_3-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}H}-CH_2-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-OH$$

Phytol

Phytol can theoretically exist in a *cis* and *trans* form, but it is not known which configuration is assumed by the natural compound. Similarly, optical activity is also possible, and indeed Willstätter and Hochheder have observed optical activity prior to distillation, although this result could not be reproduced by F. G. Fischer; Wagner-Jauregg [131] is of the opinion that natural undistilled phytol is either optically inactive or possesses only very small rotatory power. The genetic relations between phytol and carotenoids were mentioned in the section dealing with these pigments.

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[131] Wagner-Jauregg: *Z. physiol. Chem.*, 222, 21 (1933).

If chlorophyll is treated cautiously with acids, *e.g.*, oxalic acid, the magnesium is removed to give the phaeophytins, *e.g.*, phaeophytin *a*, $C_{34}H_{35}O_3N_4CO_2C_{20}H_{39}$. The phaeophytins are only weakly basic,[132] and magnesium may be reintroduced[133] with formation of chlorophyll. On treating with strong acid, the phytol residue is also split off with the formation of the phaeophorbides, *e.g.*, phaeophorbide *a*, $C_{34}H_{35}O_3N_4$-$COOH$. Like the chlorophyllides, the phaeophorbides may also be esterified in alcoholic solution yielding, for example, methyl phaeophorbide *a*, $C_{34}H_{35}O_3N_4CO_2CH_3$, and even the phytol residue[134, 135] has been reintroduced.

The compounds mentioned above all contain a second carboxyl group which is esterified with methyl alcohol, so that the formulas of chlorophyllide *a* and phaeophorbide *a* may be further resolved into

$$C_{32}H_{30}ON_4Mg(COOH)(CO_2CH_3) \text{ and } C_{32}H_{32}ON_4(COOH)(CO_2CH_3).$$

If a phaeophytin or phaeophorbide is submitted to alkaline hydrolysis, one obtains from the *a* series phytochlorin *e* (abbreviated to chlorin *e*); and from the *b* series phytorhodin *g* (or rhodin *g*), the formation[136] of which involves not only the removal of phytol and methyl alcohol, but also the opening of a ring system and the consequent appearance of still another carboxyl group; chlorin *e* thus forms a trimethyl ester[137] of the formula $C_{31}H_{33}N_4(CO_2CH_3)_3$. The name "chlorin" bears reference to the fact that it shows an olive-green color in indifferent solvents, whereas rhodin possesses a red color.

To summarize the facts so far disclosed, therefore, chlorophyll is a diester of phytol and methyl alcohol and is a complex magnesium compound which is remarkably stable toward alkali and equally remarkably sensitive toward acid reagents. Magnesium and phytol are essential features of chlorophyll and are responsible in large measure for its biochemical character. Thus phytol imparts to chlorophyll its lipoid nature and physical properties resembling those of the carotenoids, with which it is associated in the chloroplasts. The reactions of chlorophyll so far mentioned are represented in the following scheme:

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[132] The separation of chlorophyll derivatives is effected, apart from chromatographic analysis, by fractionation with hydrochloric acid. Willstätter observed that the partition relations between ether and hydrochloric acid of varying concentration vary markedly among different derivatives of chlorophyll. The acid value of a material is defined as the percentage strength of hydrochloric acid which will extract about two-thirds of that same material dissolved in an equal volume of ether. [Willstätter, Mieg: *Ann.*, 350, 1 (1906)].

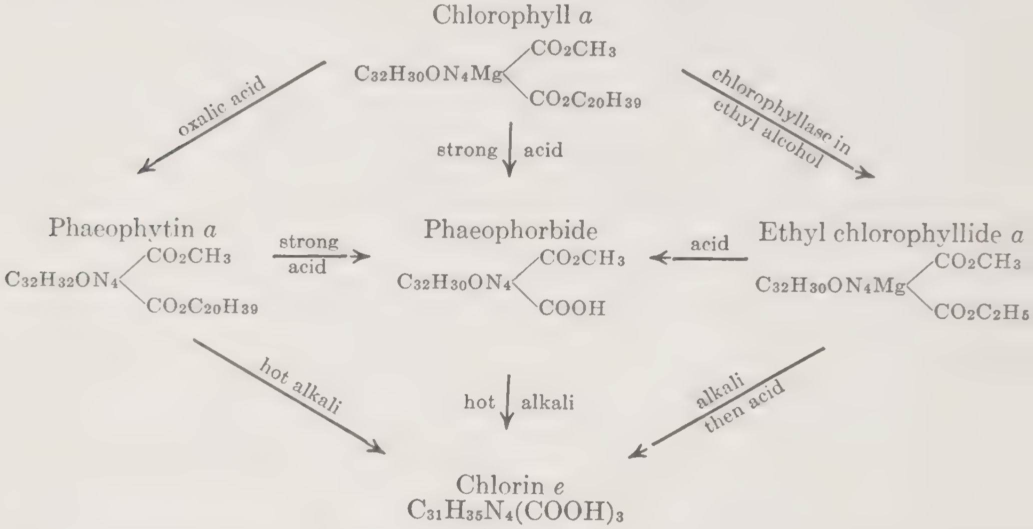
[133] Willstätter, Forsén: *Ann.*, 396, 180 (1913).

[134] Chlorophyllide *a* from phaeophorbide *a*: H. Fischer, Spielberger: *Ann.*, 510, 156 (1934).

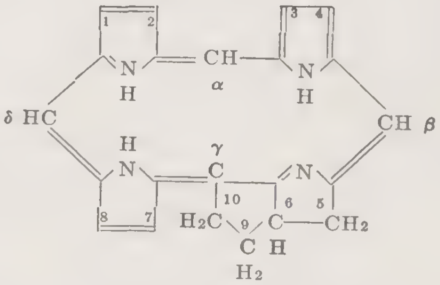
[135] H. Fischer, Schmidt: *Ann.*, 519, 244 (1935).

[136] R. Willstätter and A. Stoll: "Untersuchungen über Chlorophyll," p. 15.

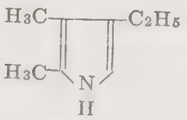
[137] H. Fischer, Herrle, Kellermann: *Ann.*, 524, 222 (1936).


Chlorophyll a
C32H30ON4Mg CO2CH3 CO2C20H39
oxalic acid
strong acid
chlorophyllase in ethyl alcohol
Phaeophytin a
C32H32ON4 CO2CH3 CO2C20H39
strong acid
Phaeophorbide
C32H30ON4 CO2CH3 COOH
acid
Ethyl chlorophyllide a
C32H30ON4Mg CO2CH3 CO2C2H5
hot alkali
hot alkali
alkali then acid
Chlorin e
C31H35N4(COOH)3

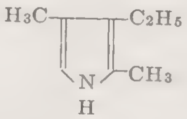
The nomenclature of chlorophyll derivatives may be referred to the accompanying structure:


1
2
3
4
N H
CH
α
N H
δ HC
CH β
H N
γ C
N
10
6
5
8
7
H2C
9
C
CH2
C H
H2

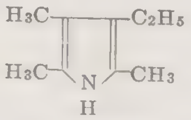
The parent structure of chlorophyll[137] is that of a dihydroporphin (phorbin), and meso-derivatives are those with an ethyl group in position 2. The chlorins are obtained by fission of the bond between C_9 and C_{10}. Now reduction[138] of chlorin *e* or ethyl chlorophyllide yields:


H3C
C2H5
H3C
N
H

Hemopyrrolle


H3C
C2H5
N
CH3
H

Kryptopyrrole


H3C
C2H5
H3C
N
CH3
H

Phyllopyrrole

Oxidation products[139] of chlorin are:

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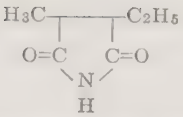
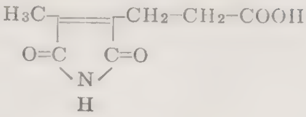
[138] Nencki, Marchlewski: *Ber.*, **34**, 1687 (1901); Willstätter, Asahina: *Ann.*, **385**, 188 (1911); H. Fischer, Merka, Plötz: *Ann.*, **478**, 283 (1930).

[139] Marchlewski: *Bull. intern. acad. sci. Cracovie*, **1902**, 1; Willstätter, Asahina: *Ann.*, **373**, 227 (1910); Treibs, Wiedemann: *Ann.*, **466**, 264 (1928).



Methylethylmaleimide



Hematic acid

The energetic action of alkali, *e.g.*, on chlorin *e*, yields a number of porphyrins [140] which are distinguished by the same prefixes as the corresponding phyllins containing magnesium.

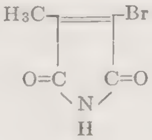
It will be appreciated that the elucidation of the constitution of chlorophyll falls into three sections:

(1) The determination of the constitution of the more simple porphyrins, a task which was facilitated by the knowledge of this group gained in the examination of hemin.

(2) The constitution of the higher porphyrins in which a further ring, the "isocyclic ring," is still intact.

(3) The determination of the cause of the isomerism existing between the higher porphyrins and the direct derivatives of chlorophyll, isomerism which is to be sought in a different disposition of the hydrogen atoms (cf. the unsaturated character of chlorophyll and its nearest derivatives).

The constitution of the simpler porphyrins will be first considered. These are produced by stepwise degradation, first verdoporphyrin,[141] then rhodoporphyrin being obtained, and at a higher temperature pyrroporphyrin and phylloporphyrin being formed. Verdoporphyrin and rhodoporphyrin are dicarboxylic acids, and pyrroporphyrin and phylloporphyrin are monocarboxylic acids. In the determination of their constitution the experience gained from the degradation of hemin was of great value. Thus oxidation [142] of brompyrroporphyrin and bromphylloporphyrin gave bromcitraconimide (I), when it was concluded that at least one pyrrole ring carries a free β-position in which rhodoporphyrin bears a carboxyl



(I)

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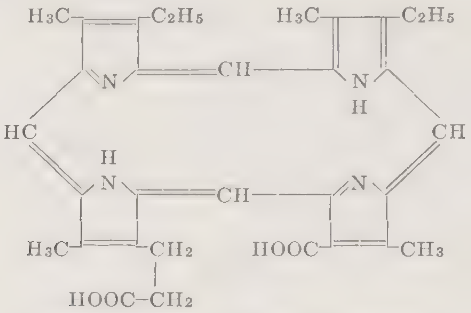
[140] Schunck: *Proc. Roy. Soc. London*, **50**, 302 (1891/2); Schunck, Marchlewski: *Ibid.*, **57**, 314 (1895); *Ann.*, **284**, 81 (1895); Willstätter, Fritzsche: *Ann.*, **371**, 33 (1909); H. Fischer, Treibs: *Ann.*, **466**, 188 (1928); Treibs, Wiedemann: *Ann.*, **466**, 264 (1928).

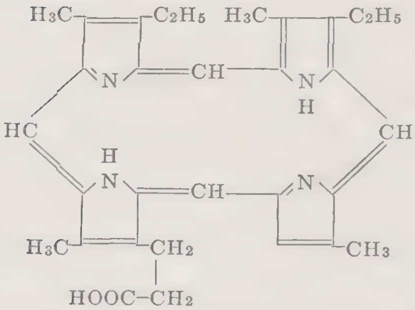
[141] Treibs, Wiedemann: *Ann.*, **471**, 146 (1929); cf. also Conant, Hyde, Moyer, Dietz: *J. Am. Chem. Soc.*, **53**, 359 (1931); Conant, Bailey: *Ibid.*, **55**, 795 (1933); H. Fischer, Klebs, Süs: *Ann.*, **490**, 44, 88 (1931).

[142] H. Fischer, Treibs: *Ann.*, **466**, 188 (1928).

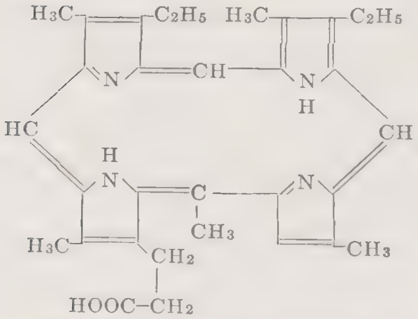
group. The constitutions have since been completely established both by degradation and by synthesis [143]:



Rhodoporphyrin, $C_{30}H_{32}N_4(COOH)_2$



Pyrroporphyrin, $C_{30}H_{33}N_4COOH$



Phylloporphyrin, $C_{31}H_{35}N_4COOH$
(*γ-methyl Pyrroporphyrin*)

The synthesis of phylloporphyrin was effected according to the following scheme [144]:

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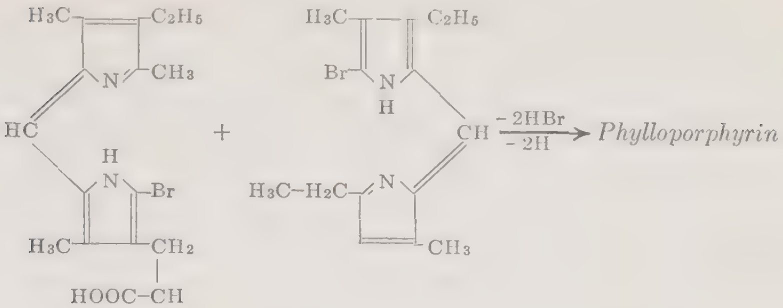
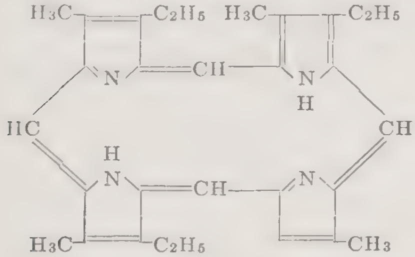
[143] H. Fischer, Hummel, Treibs: *Ann.*, **471,** 237 (1929); H. Fischer, Schormüller: *Ann.*, **473,** 211 (1929); H. Fischer, Berg, Schormüller: *Ann.*, **480,** 109 (1930); H. Fischer, Helberger: *Ann.*, **480,** 235 (1930); H. Fischer, Schormüller: *Ann.*, **482,** 232 (1930); H. Fischer, Siedel, d'Ennequin: *Ann.*, **500,** 137 (1933).

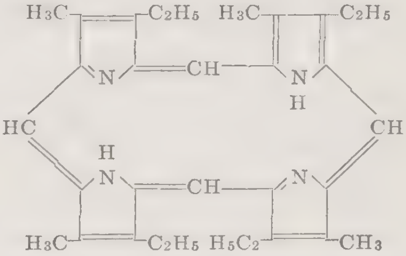
[144] H. Fischer, Helberger: *Ann.*, **480,** 235 (1930); illustrative of the difficulties encountered is the fact that 8 isomerides of tetramethyltriethylporphin monopropionic acid exist, all of which were synthesized. Each of the three ethyl groups may now theoretically be removed from each of the eight isomerides to give 24 forms of pyrroporphyrin. Synthesis of chlorophyll porphyrins: *e.g.*, H. Fischer, Böckh: *Ann.*, **516,** 177 (1935).



The propionic acid of phylloporphyrin may be decarboxylated to yield the compound (I) which is related to aetioporphyrin III [formula (II)] from the degradation of hemin from which it differs only in the lack of a β-ethyl group:

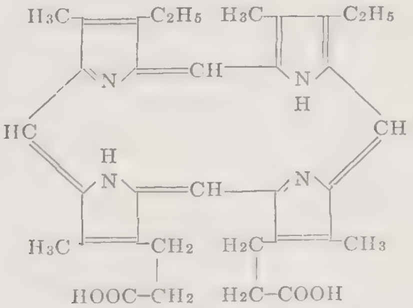


(I)



(II)

This structural relation has been confirmed by replacing the free hydrogen atom in position 6 in pyrroporphyrin by a propionic acid group and thus obtaining the mesoporphyrin of the hemin series (III) [145]:



(III)

Closer examination of some of these porphyrins has revealed the presence of vinyl groups in isorhodoporphyrin and pseudoverdoporphyrin and in chlorophyll *a* itself, and the complications ensuing on degradation of the chlorophyll molecule are often to be traced to the presence of these groups.

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[145] H. Fischer, Riedl: *Ann.*, **486**, 178 (1931); H. Fischer, Krauss: *Ann.*, **521**, 261 (1936); H. Fischer, Kahr: *Ann.*, **524**, 251 (1936).

However, the main achievement of this part of the investigation was the preparation of a porphyrin of the hemin series, thus establishing a connection [146] between the leaf and blood pigments. Thus, of the 55 carbon atoms of chlorophyll *a*, 20 belong to phytyl alcohol, one is recognized in the form of methyl alcohol, and 32 have been accounted in the determination of the constitution of phylloporphyrin, leaving two carbon atoms and the attached oxygen and hydrogen atoms to be accommodated. It remained to be proved that the porphin constitution of phylloporphyrin was also present in chlorophyll and its close derivatives, but the probability of the existence of a side-chain bound to the γ-methine bridge was certainly indicated.

Progress in the elucidation of the finer structure of chlorophyll resulted from the examination of phylloerythrin, $C_{33}H_{34}O_3N_4$, a product formed from chlorophyll in the intestinal canal of ruminants; phylloerythrin, which has also been termed choleohämatin, and bilipurpurin had been obtained by Loebisch and Fischler[147] and by Marchlewski[148] from ox-bile. H. Fischer [149] was able to obtain this difficultly accessible substance from phaeophytin, chlorophyllide and phaeophorbide, and was successful in clarifying its constitution. Its close connection with phylloporphyrin emphasized still further the close relation between chlorophyll and the porphyrins, as did also the conversion of chlorophyll derivatives into porphyrins by bacteriological means,[150] as it must be remembered that degradation to rhodoporphyrin, phylloporphyrin and pyrroporphyrin could at that time be effected only under conditions which did not exclude the possibility of secondary syntheses. Now a mild method—the action of hydriodic acid in acetic acid—is available, and with its aid phaephorbide, for example, yields phaeoporphyrins, and chlorin *e* the chloroporphyrins. Such porphyrins differ to some extent from those obtained by alkali degradation, into which, however, they may be converted. Phaeoporpyhrin a_5 is thus now known to have constitution (I) [151] and yields on hydrolysis chloroporphyrin e_6 (II) (the suffix numbers, *e.g.*, 6, refer to the number of oxygen atoms in the molecule):

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[146] Cf. also H. Fischer, Ebersberger: *Ann.*, **509,** 19 (1934).

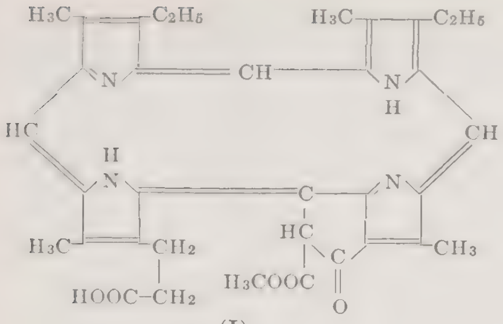
[147] Loebisch, Fischler: *Monatsh.*, **24,** 335 (1903); H. Fischer: *Z. physiol. Chem.*, **96,** 292 (1915/16).

[148] Marchlewski: *Z. physiol. Chem.*, **43,** 464 (1904/5).

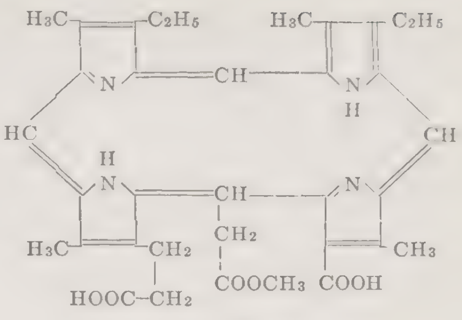
[149] H. Fischer, Filser, Hagert, Moldenhauer: *Ann.*, **490,** 1 (1931); cf. also: H. Fischer, Merka, Plötz: *Ann.*, **478,** 283 (1930); H. Fischer, Bäumler: *Ann.*, **480,** 197 (1930); H. Fischer, Süs: *Ann.*, **482,** 225 (1930); H. Fischer, Moldenhauer, Süs: *Ann.*, **486,** 107 (1931); H. Fischer, Hendschel: *Z. physiol. Chem.*, **198,** 33 (1931); **222,** 250 (1933).

[150] H. Fischer, Bäumler: *Ann.*, **474,** 65 (1929); **480,** 197 (1930); H. Fischer, Moldenhauer: *Ann.*, **478,** 54 (1930); H. Fischer, Moldenhauer: *Ann.*, **481,** 132 (1930); H. Fischer, Moldenhauer, Süs: *Ann.*, **485,** 1 (1931); **486,** 107 (1931); H. Fischer, Filser, Hagert, Moldenhauer: *Ann.*, **490,** 1 (1931).

[151] H. Fischer, Filser, Plötz: *Ann.*, **495,** 1 (1932); H. Fischer, Heckmaier, Plötz: *Ann.*, **500,** 215 (1933).

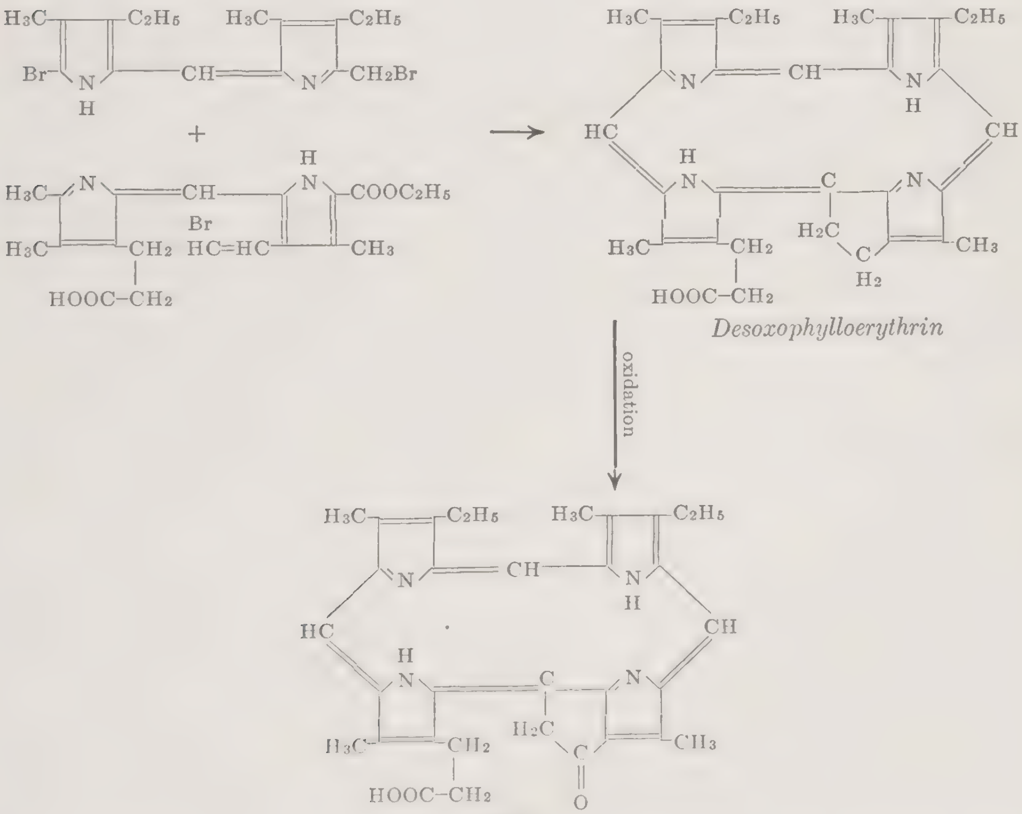


(I)



(II)

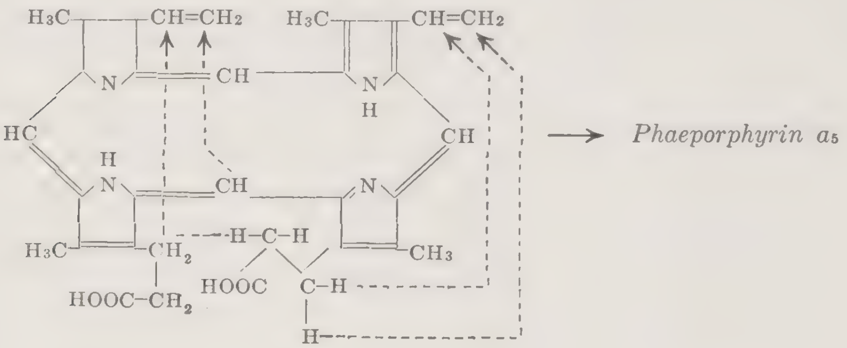
Constitution (II) may be recyclized to phaeoporphyrin a_5, decarboxylation of which yields phylloerythrin. Phylloerythrin has thus an extra isocyclic ketonic ring as in (III). This constitution has been confirmed by synthesis[152]; the ketone behaves normally and may be reduced to desoxophylloerythrin with a methylene group instead of the carbonyl group in position 9.



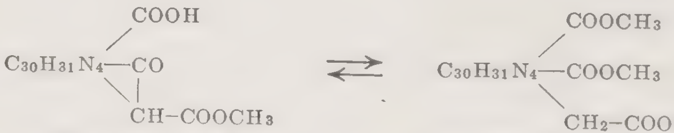
(III)

[152] H. Fischer, Riedmair: *Ann.*, **490**, 91 (1931); **497**, 181 (1932); **499**, 288 (1932); H. Fischer, Heckmaier, Riedmair: *Ann.*, **494**, 86 (1932); H. Fischer, Speitmann, Meth: *Ann.*, **508**, 154 (1934); H. Fischer, Müller, Leschhorn: *Ann.*, **523**, 165 (1936); H. Fischer, Kellermann: *Ann.*, **524**, 25 (1936).

Phaeoporphyrin a_5 may thus be regarded as a derivative of protoporphyrin in which the propionic residue in position 6 has been oxidized to a β-ketopropionic residue, cyclization has taken place between the α-carbon atom of this propionic residue and the γ-methine bridge, and the two vinyl groups have become reduced to ethyl groups. Formally, therefore, phaeoporphyrin a_5 is a monoxide of protoporphyrin:



It may be remarked that the individual form in which the above formulas are written with two diagonally opposite nuclei having a pyrrolenine structure[153] is favored by spectroscopic evidence. It will be recalled that phaeoporphyrin a_5[154] is formed by reducing phaeophorbide, or even chlorophyll or a chlorophyllide, with hydrogen iodide in acetic acid, just as chloroporphyrin e_6 is obtained from chlorin e. It is logical to conclude, therefore, that the isocyclic ring present in phaeoporphyrin a_5 also exists in phaeophorbide, and that chlorin e must contain a side-chain formed by fission of the isocyclic ring and corresponding to that of chloroporphyrin e_6. Indeed, phaeophorbide may be converted into chlorin e_6 trimethyl ester, and the reverse reaction has also been effected:



$$\mathrm{C_{30}H_{31}N_4}\begin{array}{l}\diagup\mathrm{COOH}\\ -\mathrm{CO}\\ \diagdown\ \ \mid\\ \quad\mathrm{CH{-}COOCH_3}\end{array} \rightleftarrows \mathrm{C_{30}H_{31}N_4}\begin{array}{l}\diagup\mathrm{COOCH_3}\\ -\mathrm{COOCH_3}\\ \diagdown\\ \quad\mathrm{CH_2{-}COOCH_3}\end{array}$$

Phaeophorbide a *Trimethyl ester of chlorin e_6*

The synthesis of the isocyclic ring has recently been achieved in the chlorin series also; thus a partial synthesis of methylphaeophorbide *a* from chlorin e_6 trimethyl ester has been carried out.[154a]

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[153] Stern, Wenderlein: *Z. physik. Chem.*, **175A**, 429 (1936).

[154] H. Fischer, Lakatos: *Ann.*, **506**, 148 (1933); H. Fischer, Riedmair, Hasenkamp: *Ann.*, **508**, 248 (1934); H. Fischer, Spielberger: *Ann.*, **510**, 166 (1934).

[154a] Fischer, Oestreicher, *Ann.*, **546**, 49 (1940).

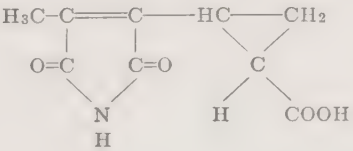
Similarly the same isocyclic ring must be assumed also in chlorophyll [155] itself.

The facts given above do not completely explain, however, the connection between the phorbides and the porphyrins. Both chlorophyll and phaeophorbide possess a readily hydrogenated double bond.[156] Phaeophorbide may thus be converted into dihydrophaeophorbide, and chlorin e_6 into dihydrochlorin e_6. If the reduction of phaeophorbide is carried out beyond the dihydro-stage, the new compound can be oxidized by air to yield phaeoporphyrin a_5:

$$\underset{C_{35}H_{36}O_5N_4}{\text{Phaeophorbide } a} \xrightarrow{+H_2} \underset{C_{35}H_{38}O_5N_4}{\text{Dihydrophaephorbide}} \xrightarrow{+2H_2}$$

$$\underset{C_{35}H_{42}O_5N_4}{\text{Phaeoporphyrinogen } a_5} \xrightarrow{-3H_2} \underset{C_{35}H_{36}O_5N_4}{\text{Phaeoporphyrin } a_5}$$

Phaeophorbide a and phaeoporphyrin a_5 on the one hand, and chlorin e_6 and chloroporphyrin e_6 on the other have the same carbon, oxygen and nitrogen content, and that their hydrogen contents are also equal, as is indicated above, is evidenced by calorimetric measurements.[157] The explanation of the isomerism between these pairs of compounds lies in the fact that the unsaturated isomerides contain a vinyl group and that the two hydrogen atoms must be accommodated elsewhere in the molecule. The presence of the vinyl group has been verified by its reaction with diazoacetic ester.[158] Pyrroles containing unsaturated side-chains react directly with diazoacetic ester, and thus protoporphyrin and hemin yield adducts which upon oxidation give, among other products, methylmaleimidocyclopropyl carboxylic acid:



Methylphaeophorbide a undergoes the same reaction, and the adduct affords the same oxidation product, so that it is evident that methylphaeophorbide a also contains a vinyl group [159] in position 2. Incidentally, the recognition of this vinyl group provides an explanation of the so-called oxoreaction,[160] whereby ketoporphyrins are obtained by the action of colorless hydriodic acid in cold acetic acid solution. Hydriodic acid is first added to the vinyl group, the iodine atom is exchanged for hydroxyl, and spontaneous dehydrogenation then ensues with the formation of an acetyl residue according to the following scheme:

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[155] Conant, Hyde: *J. Am. Chem. Soc.*, **51**, 3668 (1929); H. Fischer, Filser, Hagert, Moldenhauer: *Ann.*, **490**, 31 (1931).

[156] Conant, Hyde: *J. Am. Chem. Soc.*, **51**, 3668 (1929); H. Fischer, Helberger: *Ann.*, **480**, 249 (1930); Stoll, Wiedemann: *Naturwiss.*, **20**, 791 (1932); H. Fischer, Lakatos: *Ann.*, **506**, 123 (1933); H. Fischer, Lakatos, Schnell: *Ann.*, **509**, 201 (1934).

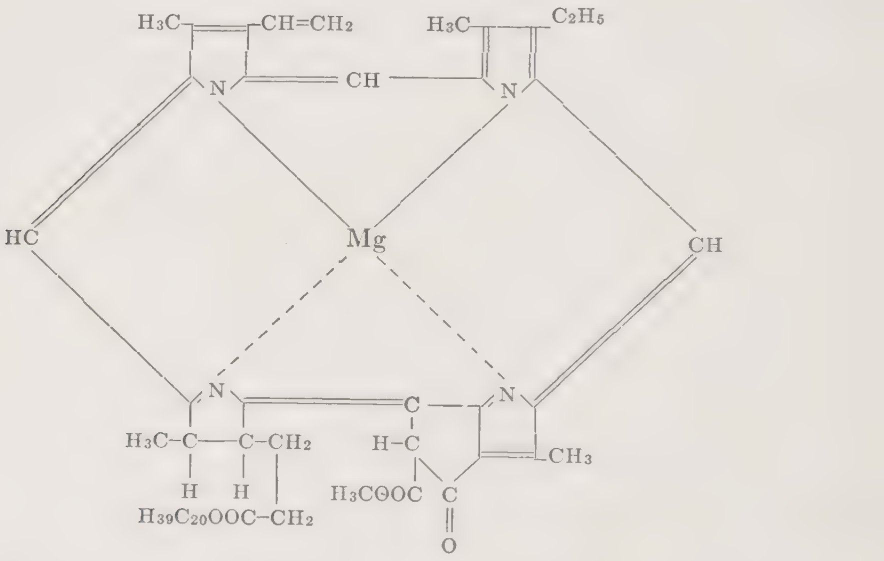
[157] Stern, Klebs: *Ann.*, **500**, 91 (1933); **504**, 287 (1933); **505**, 295 (1933); cf. also H. Fischer, Süs, Klebs: *Ann.*, **490**, 38 (1931); H. Fischer, Siebel: *Ann.*, **494**, 73 (1932).

[158] H. Fischer, Staff: *Z. physiol. Chem.*, **234**, 97 (1935); H. Fischer, Medick: *Ann.*, **517**, 246 (1935).

[159] H. Fischer, Hasenkamp: *Ann.*, **513**, 111 (1934); H. Fischer, Böckh: *Ann.*, **516**, 177 (1935); H. Fischer, Rose: *Ann.*, **519**, 1 (1935); H. Fischer, Krauss: *Ann.*, **521**, 267 (1936); H. Fischer, Wunderer: *Ann.*, **533**, 230 (1938).

$$-CH{=}CH_2 \xrightarrow{HI} -CHI{-}CH_3 \longrightarrow -CH(OH)CH_3 \xrightarrow{\text{Dehydrogenation}} -COCH_3$$

The green phorbides and chlorins are thus structurally distinguished from the red porphyrins by the presence of a vinyl group and of two additional hydrogen atoms, for which accommodation must be found in the chromophoric system and which are easily removed, as in the dehydrogenation of phaeophorbide.[161] They must indeed be placed in one of the pyrrole nuclei, to which they would impart a hydroaromatic character; earlier formulas would now seem to be overthrown by the observation that among the oxidation products of chlorophyll itself no hematic acid [162] is to be found and hence the following constitution is now suggested (1939):



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[160] H. Fischer, Riedmair: *Ann.*, **505,** 87 (1933); H. Fischer, Riedmair, Hasenkamp: *Ann.*, **508,** 224 (1934); H. Fischer, Hasenkamp: *Ann.*, **513,** 107 (1934); H. Fischer, Rose: *Ann.*, **519,** 1 (1935).

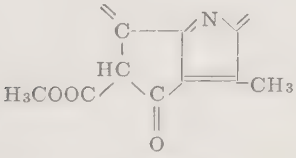
[161] H. Fischer, Lautsch: *Ann.*, **525,** 259 (1936); **528,** 247 (1937); cf. H. Fischer, Herrle: *Ann.*, **527,** 138 (1937).

[162] H. Fischer, Wenderoth: *Ann.*, **537,** 170 (1939). Earlier formulas: H. Fischer, Siebel: *Ann.*, **499,** 90 (1932); H. Fischer: *Ann.*, **502,** 175 (1933); H. Fischer, Riedmair, Hasenkamp: *Ann.*, **508,** 224 (1934); H. Fischer, Hasenkamp: *Ann.*, **513,** 107 (1934); Stoll, Wiedemann: *Helv. Chim. Acta*, **17,** 163 (1934); earlier work of Stoll, Wiedemann: *Naturwiss.*, **20,** 706, 791 (1932); *Helv. Chim. Acta*, **15,** 1128, 1280 (1932); **16,** 183, 307, 739 (1933); **17,** 456, 837 (1934); cf. also Armstrong: *J. Soc. Chem. Ind.*, **52,** 809 (1933); Conant, Dietz, Bailey, Kamerling: *J. Am. Chem. Soc.*, **53,** 2382 (1931); Conant, Dietz: *Nature*, **131,** 131 (1933).

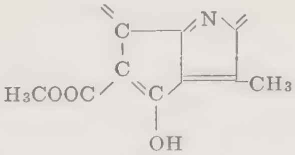
It is improbable that any extensive alterations in this formula will be necessitated. It will be observed that the deduction of this formula takes no account of the spectroscopic observations of Stern in regard to the pyrrolenine structure, but on the other hand Stern's conclusions concern the porphyrin rather than the dihydroporphin system.

Clearly, as in the porphyrins, the aromatic character is due to the conjugation of double bonds. The presence of an asymmetric carbon atom—the asymmetric center is located at C_{10}—gives rise to optical activity[163]; $[\alpha]\frac{25}{720}$ for chlorophyll *a* is $-260°$ ($\pm 25°$) but racemization is rapid. The apparent optical inactivity of some chlorophyll derivatives is probably due to strong rotatory dispersion.[163a]

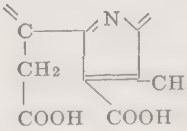
No mention has yet been made of the so-called phase test and allomerization which have played a part in these investigations. The natural mixture of chlorophylls *a* and *b* undergoes a transient color change to brown on shaking with concentrated methyl alcoholic caustic potash[164]; the pure *a* component at this stage is yellow, and the *b* compound is red. After a few minutes the original color returns. The appearance of the brown phase is associated with the presence of the unchanged isocyclic ring and marks its hydrolytic fission. The grouping is indeed that of a substituted acetoacetic ester which can undergo enolization; the green color returns with the inception of "acid hydrolysis," but the absence of the β-ketoester grouping precludes a second appearance of the brown phase:



Green



Brown
a—yellow
b—red



Green

If the enolate formation is quickly interrupted the process can be reversed and unchanged chlorophyll is recovered. The phase test is thus a sensitive means of establishing the intact nature of the isocyclic ring of chlorophyll.

Ethyl alcoholic solutions of chlorophyll and its near derivatives readily undergo a change, "allomerization," after which the brown phase can no longer be observed. Allomerized products are more easily soluble, are purified and crystallized only with the greatest difficulty, and on alkaline hydrolysis and fractionation yield much more weakly basic chlorins and rhodins. It was found by Conant[165] that one molecule of oxygen is utilized during allomerization, and the reaction is thus fundamentally one of oxidation. The action of hydriodic acid in acetic acid on allomerized products[166] yields first porphyrins which, instead of the original isocyclic ring, possess the grouping:

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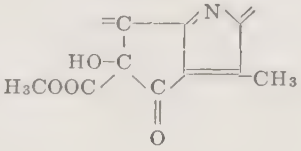
[163] Stoll, Wiedemann: *Helv. Chim. Acta*, **16**, 307 (1933); H. Fischer, Bub: *Ann.*, **530**, 213 (1937).

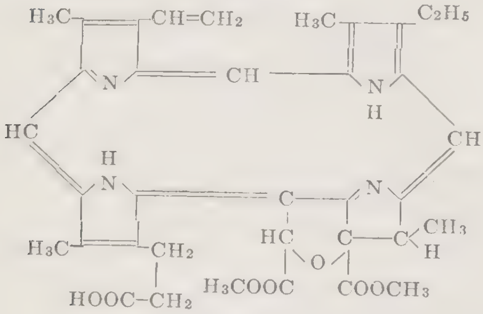
[163a] Pruckner, Oestreicher, Fischer: *Ann.*, **546**, 41 (1940).

[164] R. Willstätter and A. Stoll, "Untersuchungen über Chlorophyll," pp. 144, 145; Willstätter, Utzinger: *Ann.*, **382**, 129 (1911); Willstätter, Stoll: *Ann.*, **387**, 317 (1912), particularly p. 357.

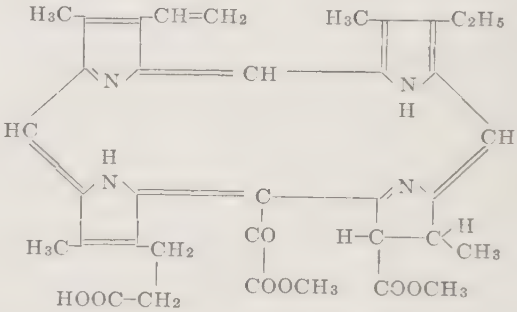


More recently formulas for these allomerized products with peroxide structures have been discussed and model experiments to replace the oxygen used in the allomerization process by benzoquinone as the acceptor have also been described.

The so-called purpurins[167] deserve reference in view of their close relation to allomerized chlorophyll. They are compounds possessing constitutions formerly believed to be of type (I); according to a recent publication H. Fischer suggests formula (II):



(I)



(II)

Purpurin 7

Purpurins are formed by the action of sodium propoxide on chlorophyll, phaeophorbides and chlorins. They can be converted into chlorins[168] such as rhodochlorin, pyrrochlorin and phyllochlorin:

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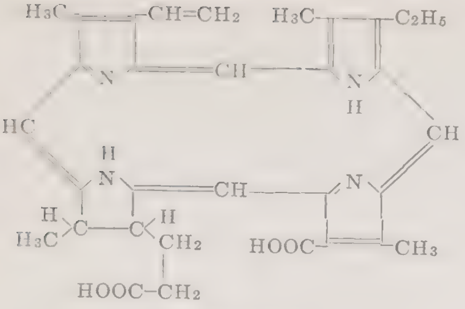
[165] Conant, Moyer: *J. Am. Chem. Soc.*, **52**, 3013 (1930); Conant, Kamerling, Steele: *Ibid.*, **53**, 1615 (1931); Steele: *Ibid.*, **53**, 3171 (1931).

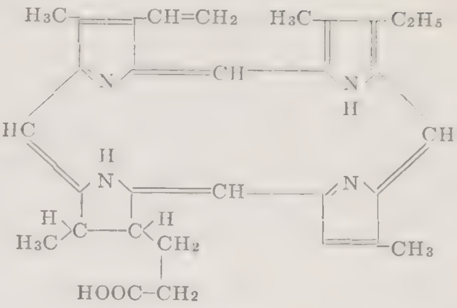
[166] H. Fischer, Filser, Plötz: *Ann.*, **495**, 1 (1932); H. Fischer, Riedmair: *Ann.*, **497**, 181 (1932); H. Fischer, Heckmaier, Plötz: *Ann.*, **500**, 215 (1933); H. Fischer, Hagert: *Ann.*, **502**, 41 (1933); H. Fischer, Riedmair: *Ann.*, **505**, 87 (1933); **506**, 107 (1933); H. Fischer, Hasenkamp: *Ann.*, **513**, 107 (1934), particularly p. 115.

[167] Marchlewski: *Biochem. Z.*, **277**, 171 (1935); Conant, Moyer: *J. Am. Chem. Soc.*, **52**, 3013 (1930); Conant, Hyde, Moyer, Dietz: *Ibid.*, **53**, 362 (1931); H. Fischer, Lautsch: *Ann.*, **528**, 253 (1937); H. Fischer, Kahr: *Ann.*, **531**, 209 (1937).

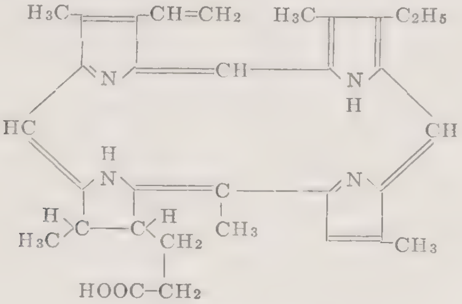
[168] The location of the two additional hydrogen atoms is still uncertain.


H₃C
CH=CH₂
H₃C
C₂H₅
N
CH
N
H
HC
CH
H
N
CH
N
H
H₃C
C
C
H
CH₂
HOOC
CH₃
HOOC–CH₂

Rhodochlorin


H₃C
CH=CH₂
H₃C
C₂H₅
N
CH
N
H
HC
CH
H
N
CH
N
H
H₃C
C
C
H
CH₂
CH₃
HOOC–CH₂

Pyrrochlorin


H₃C
CH=CH₂
H₃C
C₂H₅
N
CH
N
H
HC
CH
H
N
C
N
H
H₃C
C
C
H
CH₂
CH₃
CH₃
HOOC–CH₂

Phyllochlorin

The phytyl group is placed on the propionic acid residue [169] of pyrrole nucleus IV, as is shown in the formula of chlorophyll above. Proof of this resides in the fact that phylloerythrin and its corresponding pyrrophaeophorbide *a* are formed when phaeophorbide *a* is heated in pyridine. The carbomethoxy group which is lost is therefore attached to the isocyclic ring and there remains only the propionic residue to accommodate the phytyl alcohol. Even more convincing is the reduction of ethyl chlorophyllide α by hydriodic acid. The ethyl ester of phaeoporphyrin α_5, which is first formed, is then pyrolyzed to yield phylloerythrin ethyl ester and not the methyl ester, which would have been obtained if chlorophyll had been a methyl and not a phytyl propionic ester.

Chlorophyll *b*.[170] The formula of chlorophyll *b* differs from that of the *a* compound by containing two less hydrogen atoms and one more atom of oxygen. Degradation gives the same porphyrins, and the disposition of the isocyclic ring is similar in the two compounds; characteristic of chlorophyll *b*, however, is an aldehydic group, the position of which has been ascertained in the following manner. The appropriate phorbide, phaeophorbide b_6, was converted into rhodoporphyrin g_7 which still contained the aldehydic group and was therefore oxidized to the carboxylic acid. This was then recyclized to the phorbide type, phaeophorbide b_7, and by reduction and decarboxylation was converted into a derivative resembling phylloerythrin. The latter was identified by synthesis as 3-desmethyldesoxophylloerythrin, and it is clear that it is the vacant 3-position which must originally have been occupied by the aldehyde group:

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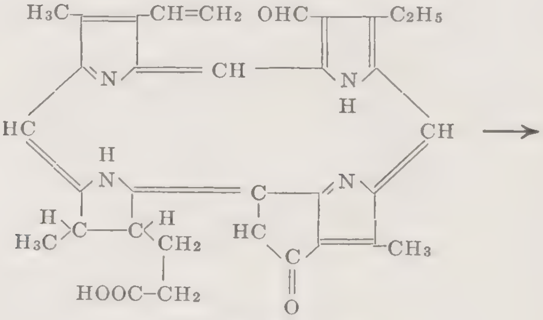
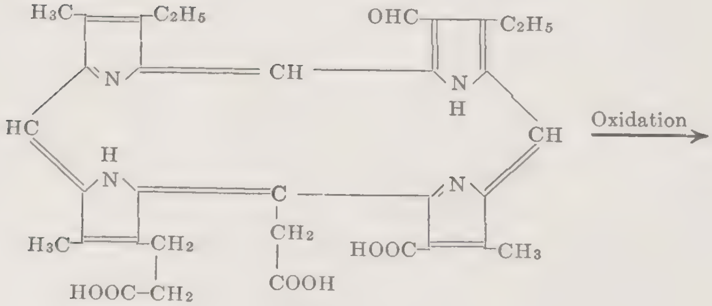
[169] Conant, Hyde: *J. Am. Chem. Soc.*, **51**, 3668 (1929); Conant, Dietz, Bailey, Kamerling: *Ibid.*, **53**, 2382 (1931); H. Fischer: *Ann.*, **502**, 175 (1933) and p. 197; cf. however, Stoll, Wiedemann: *Helv. Chim. Acta*, **17**, 163 (1934), particularly p. 167.

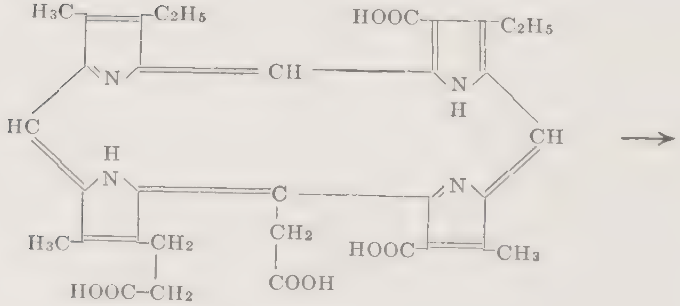
[170] R. Willstätter and A. Stoll: "Untersuchungen über Chlorophyll"; Treibs, Wiedemann: *Ann.*, **471**, 146 (1929); Conant, Dietz, Werner: *J. Am. Chem. Soc.*, **53**, 4436 (1931); Warburg, Christian: *Biochem. Z.*, **235**, 240 (1931); Warburg, Negelein: *Biochem. Z.*, **244**, 9 (1932); Stoll, Wiedemann: *Helv. Chim. Acta*, **15**, 1280 (1932); H. Fischer, Broich, Breitner, Nüssler: *Ann.*, **498**, 228 (1932); H. Fischer, Breitner, Hendschel, Nüssler: *Ann.*, **503**, 1 (1933); H. Fischer, Hendschel, Nüssler: *Ann.*, **506**, 83 (1933); H. Fischer, Riedmair, Hasenkamp: *Ann.*, **508**, 224 (1934) and p. 226; H. Fischer, Breitner: *Ann.*, **510**, 183 (1934); **511**, 183 (1934); cf. also Stoll, Wiedemann: *Helv. Chim. Acta*, **17**, 456 (1934); H. Fischer, Hasenkamp: *Ann.*, **513**, 107 (1934), and p. 114; H. Fischer, Spielberger: *Ann.*, **515**, 130 (1935); H. Fischer, Breitner: *Ann.*, **516**, 61 (1935); H. Fischer, Grassl: *Ann.*, **517**, 1 (1935); H. Fischer, Bauer: *Ann.*, **523**, 235 (1936); H. Fischer, Lautenschlager: *Ann.*, **528**, 9 (1937); H. Fischer, Lautsch: *Ann.*, **528**, 265 (1937). Proof of the position of the aldehyde group: H. Fischer, Breitner: *Ann.*, **516**, 61 (1935).

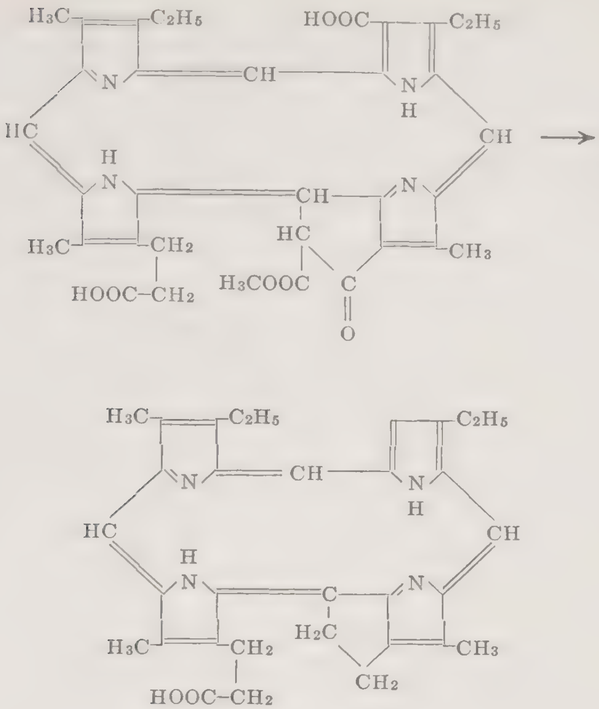


Phaeophorbide b_6



Rhodoporphyrin g_7





3-Desmethyldesoxophylloerythrin

Total synthesis[171] of chlorophyll has not yet been effected; but as described in the foregoing pages, simple porphyrins and also higher porphyrins up to desoxophylloerythrin have been obtained synthetically. Chlorophylls *a* and *b* are obtainable from chlorin e_6 and rhodin g_7 respectively, but there is still a gap between the porphyrins and chlorin e_6 or rhodin g_7.

Comparatively little is known of the mechanism of photosynthesis with which chlorophyll is so intimately connected. It is believed to comprise two coupled processes, one the photoreaction, a transformation of energy, and the other, the Blackmann reaction, an enzymatic process. The necessity of chlorophyll in the second reaction is not indisputable but its participation in the first is undoubted. It is surprising that chlorophyll, when in solution and freed from association with the leaf, is easily destroyed by light whereas it is unusually stable in the leaf. Chlorophyll is associated in the chloroplast with colloidal protein, and chloroplastin solutions have been prepared from chlorophyll and carrier. Chloroplasts are composed of 80 per cent protein, 10-20 per cent lipoid material and 5 per cent pigment, but all experiments to reproduce the assimilatory process with the aid of isolated chloroplasts have given only negative results.

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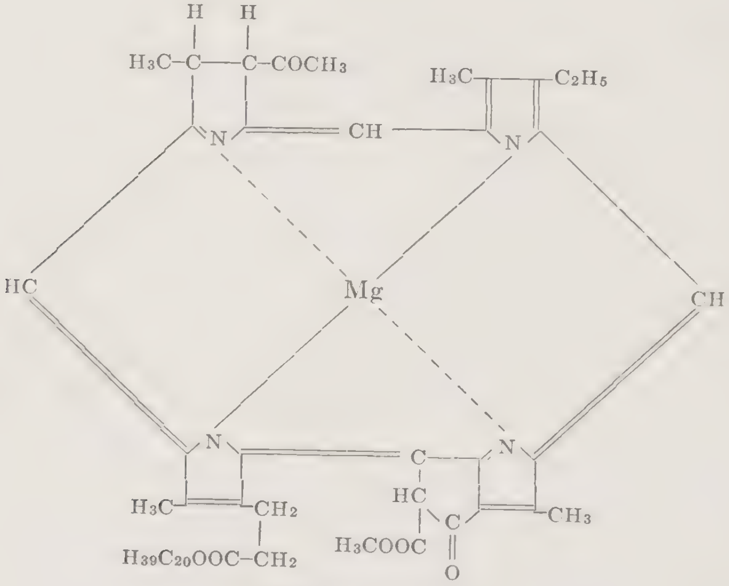
[171] Literature: A. Stoll and E. Wiedemann: "Fortschritte der Chemie organischer Naturstoffe," Berlin, 1938.

Protochlorophyll.[172] This term connotes a green pigment found in etiolated plants and seed cases. It consists of two compounds *a* and *b*, and contains magnesium in a complex form. After removing magnesium, the chief component is identical [173] with vinyl-phaeoporphyrin a_5. Protochlorophyll is identical with the material formerly termed trichosanthin.[173a]

Phyllobombicin,[174] $C_{34}H_{36}O_6N_4$, was obtained from the excrement of the silk-worm, *Bombyx mori.* It has since been suggested, however, that phyllobombicin is identical with phaeophorbide *a*.

Bacteriochlorophyll (formerly termed bacteriochlorin).[175] This is the chief pigment of the *Thio-* and *Athio-rhodaceae* (*purpurbacteria*). It



Bacteriochlorophyll

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[172] Monteverde, Lubimenko: *Bull. Akad. Imp. Sci. Petrograd (6)*, **5**, 73 (1911); **6**, 607 (1912); Lubimenko, Gortikowa: *J. Botan. Inst. W.U.A.N.*, **1934**, No. 9; Monteverde: *Acta Horti Petrop.*, **13**, II, 201 (1894); Noack: *Naturwiss.*, **17**, 104 (1929); Noack, Kiessling: *Z. physiol. Chem.*, **182**, 13 (1929); 193, 97 (1930); *Angew. Chem.*, **44**, 93 (1931); Rothemund: *Cold Spring Harbor Symposia on Quant. Biol.*, **III**, 71 (1935); Seybold: *Planta*, **26**, 712 (1937); Seybold, Egle: *Planta*, **29**, 119 (1939).

[173] H. Fischer, Mittenzwei, Oestreicher: *Z. physiol. Chem.*, **257**, IV (1939).

[173a] Seybold, *Sitz.-Ber. Heidelberger Akad. Wiss.*, **1939**, 3.

[174] H. Fischer, Hendschel: *Z. physiol. Chem.*, **198**, 33 (1931); **206**, 255 (1932); **222**, 250 (1933); Oku: *Bull. Agri. Chem. Soc. Japan*, **10**, 158, 164 (1934); **11**, 8, 9, 10 (1035) have established the presence of a glycoside, $C_{20}H_{19}O_7N$, which possesses, apart from the sugar hydroxyl groups, 3 hydroxyl groups and the character of a flavone compound: cf. Jucci, Manunti: *Atti Accad. Lincei (6)*, 15, 473 (1932); Jucci: *Boll. soc. biol. sper.*, **7**, 163 (1934).

[175] H. Fischer, Riedmair, Hasenkamp: *Ann.*, **508**, 236 (1934); H. Fischer, Hasenkamp: *Ann.*, **515**, 148 (1935); H. Fischer, Lambrecht: *Ann.*, **523**, 249 (1936); H. Fischer, Lambrecht, Mittenzwei: *Z. physiol. Chem.*, **253**, 1 (1938); cf. H. Fischer, Lambrecht: *Ibid.*, **249**, I (1937); French: *Science*, 88, 60 (1938); van Niel, Arnold: *Enzymologia*, **5**, 244 (1938); Schneider: *Chem. Zentr.*, **1936**, I, 3525. Proof that hematic acid is found on oxidative degradation is still lacking. Seybold, Egle, *Sitz.-Ber. Heidelberger Akad. Wiss.*, **1939**, 7.

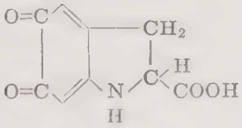
possesses the formula $C_{55}H_{74}O_6N_4Mg$, but experiments have shown that it consists of an *a* component and a very small amount of a *b* component. The same kind of method as served for the elucidation of the constitution of chlorophyll has been applied to bacteriochlorophyll, with the result that it is now known that the bacterial pigment contains an acetyl group in place of the vinyl group of chlorophyll and two more atoms of hydrogen beyond the additional ones of chlorophyll. The foregoing formula is therefore suggested.

Bacterioviridin,[176] the pigment of green bacteria, seems to be intermediate between chlorophyll *a* and bacteriochlorophyll in containing merely an acetyl group in place of the vinyl group in position 2 of chlorophyll. The pigment of purple sulfur bacteria[177] *Beggiata thiocystis* (Gaffron) is a porphyrin spectroscopically identical with oxyphaeoporphyrin.

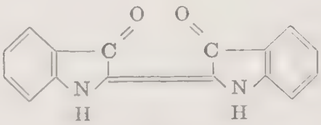
Bonelline, needles, m.p. $C_{34}H_{36\pm 2}O_4N_4$, is obtained from an alcoholic extract of the worm *Boniellia viridis.* It possesses basic properties, gives blue solutions in acids, and gives complexes with iron, copper, or zinc salts. The similarity of its absorption and fluorescence spectra to those of mesopyrrochlorin seem to warrant its inclusion among the pyrrole pigments.[177a]

Derivatives of Indole

A red coloring matter[178] is present in the epidermis of the worm *Halla parthenopaea Costa.* Of the formula $C_9H_4O_7N$, it yields a pyrroline on distillation with soda-lime, and pyrrole tricarboxylic acid on oxidation with hydrogen peroxide. A hydrazone has been obtained with *p*-bromphenylhydrazone, and the author proposes the following formula (I):



(I)



(II)

The chromogen of human melanuria[179] is probably a derivative of 5,6-dihydroxyindole.

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[176] Metzner: *Ber. deut. botan. Ges.*, 40, 125 (1922); H. Fischer, Lambrecht, Mittenzwei: *Z. physiol. Chem.*, 253, 1 (1938).

[177] H. Fischer, Riedmair, Hasenkamp: *Ann.*, 508, 224 (1934), particularly p. 236.

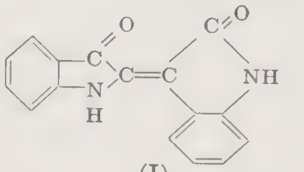
[177a] Lederer, *Compt. rend.*, 209, 528 (1939).

[178] Mazza, Stolfi: *Arch. sci. biol. (Italy)*, 16, 183 (1931); Friedheim: *Biochem. Z.*, 259, 257 (1933); the formation of dimethoxyindole by reduction with aqueous sulfurous acid—described by Mazza—is difficult to understand.

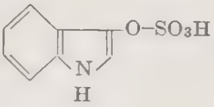
[179] Linnell, Raper: *Biochem. J.*, 29, 76 (1935).

Indigo (II), $C_{16}H_{10}O_2N_2$, occurs as the glucoside indican,[180] $C_{14}H_{17}O_6N$ (needles, m.p. 180°), in various species of *Indigofera* (*Papilionatae*), as in *Indigofera tinctoria, pseudotinctoria, anil, disperma,* and *argentea.* The plant was originally indigenous to the region between latitudes 20-30° of eastern Asia, but it thrives also in China, Japan, the Philippines, Central America, Brazil and Java. Common woad, *Isatis tinctoria* (family of *Cruciferae*) was formerly cultivated in Europe but yields a smaller amount of indican than *Indigofera;* and Dyer's knotgrass, *Polygonum tinctorium,* which also contains indican, was at one time grown in China and the Caucasus. Many other plants, such as the *oleander Nerium tinctorium* and *Lonchocarpus cyanescens* ("gara") from Sierra Leone and the western Sudan, contain indican. Bengal indigo is the most important brand, but the finest material comes from Java.

Natural indigo contains as impurity from 2 to 4 per cent (East Indies varieties) of indirubin (indopurpurin or indigo red) (I) $C_{16}H_{10}O_2N_2$, and Java products sometimes contain as much as 15 per cent.



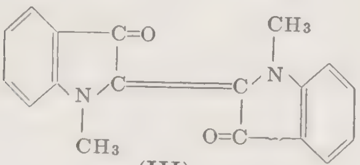
(I)



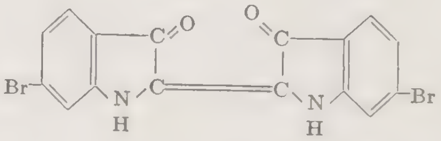
(II)

Urine indican,[181] found in the urine of mammals, is the potassium salt of indoxyl sulfuric arid (II). It probably arises by the hydroxylation of indole formed during intestinal decomposition, and the indoxyl is then bound to sulfuric acid.[182]

Indigo green,[183] which has been isolated from human urine, is assumed to possess structure (III):



(III)



(IV)

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[180] Historical: v. Georgievics: "Der Indigo vom praktischen und theoretischen Standpunkt," Leipzig and Vienna, Franz Deuticke, 1892; details of culture and isolation: Rawson: *J. Soc. Chem. Ind.*, 18, 467 (1899); van Lookeren-Campagne: "Plantagenindigo Wageningen," 1901; Schultz, "Farbstofftabellen," 7th ed., Vol. **I,** No. 1384, p. 642; Ullmann, "Encyclopaedie der technischen Chemie," 2nd ed., Vol. **VI,** p. 244; A. G. Perkin and A. E. Everest, "Natural Organic Colouring Matters"; detection and preparation of indican: Rosenthaler in Klein, "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 1060; distribution and occurrence of the glycosides: Hadders in Klein, "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 1062; Bolten: *Dyer,* 73, 217 (1935); constitution, etc.: Fritz Mayer, "Chemie der organischen Farbstoffe," 3rd ed., Berlin, 1934.

[181] Literature: V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," Vol. **II,** 3, p. 247; Stanford considers it doubtful whether indican of human urine has the above composition [*Z. physiol. Chem.* **87,** 188, 198 (1913)].

[182] Literature: *Ibid.*, p. 248.

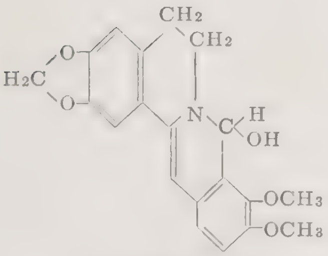
[183] Benedicenti: *Z. physiol. Chem.*, **53,** 181 (1907); Rastelli: *Arch. intern. Pharmacodynamie,* **40,** 482 (1931).

Tyrian purple,[184] $C_{16}H_8O_2N_2Br_2$. *Murex brandaris,* found on Mediterranean coasts, provides a glandular secretion which on irradiation yields 6,6′-dibromindigo (IV), the chief constituent of Tyrian purple (Friedländer). *Murex trunculus* also contains the same compound accompanied by a small amount of a similar but blue-violet product; the hypothesis that this consists of N-methyl derivatives [185] of dibromindigo has not yet been substantiated. *Purpura haemostoma* [186] from the Phoenician coast, and *Purpura aperta* (Blainv.) from the Mexican coast, also yield the same compound (although identification was not possible with absolute certainty in the latter case); and the same is true of another European purple snail, *Purpura lapillus,*[187] frequently found on the rocky coasts of Brittany, Wales, and Norway. In no case has the nature of the glandular precursor of the pigment yet been established. Dyeings with dibromindigo are red-violet but are not of an especially pleasing shade. 12,000 individuals of *Murex brandaris* yielded 1.4 grams of pigment.

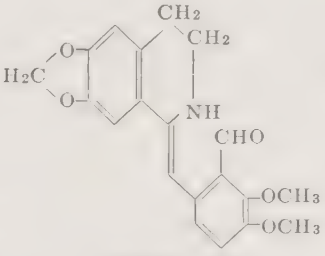
Haliotis indigo.[188] The limpet *Haliotis californiensis* contains a blue-green coloring matter believed to be constitutionally closely related to indigo.

Derivatives of Pyridine

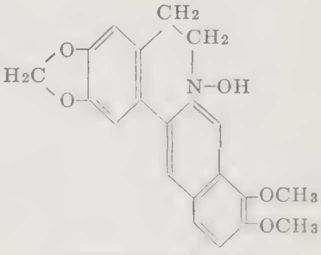
Berberine,[189] $C_{20}H_{19}O_5N$ (yellow needles, m.p. 144°), occurs in the roots of the barberry, *Berberis vulgaris,* in *Hydrastis canadensis,* one of the American *Ranunculaceae,* and in many other plants. Berberine base is unstable and assumes the aldehyde form berberinal, whereas its salts



Carbinol form



Aldehyde form



Ammonium form

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[184] A. Dedekind: "Ein Beitrag zur Purpurkunde"; Schultz: "Farbstofftabellen," 7th ed., Vol. **I,** No. 1383, p. 641; Friedländer: *Monatsh.,* **28,** 991 (1907); *Ber.,* **42,** 765 (1909); **55,** 1655 (1922); **41,** 1035 (1908).

[185] Ettinger, Friedländer: *Ber.,* **45,** 2074, 2081 (1912).

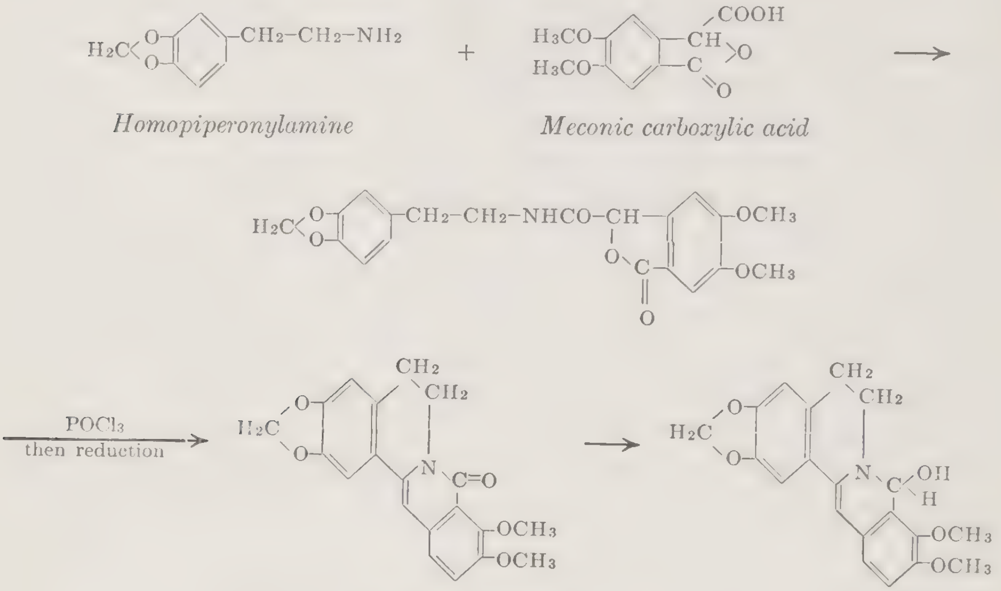
[186] Lacaze-Duthiers: *Arch. Zool. (3),* 4 (1896).

[187] Earlier work: Letellier: *Compt. rend.,* **109,** 82 (1899); **111,** 307 (1890); Schunck: *J. Chem. Soc.,* **35,** 589 (1879); Friedländer: *Ber.,* **55,** 1655 (1922).

[188] Krukenberg: *Centr. med. Wis.,* **21,** 785 (1883); Schulz: *Z. allg. Physiol.,* **3,** 91 (1904); Schulz, Becker: *Biochem. Z.,* **236,** 99 (1931); cf. Lemberg: *Z. physiol. Chem.,* **200,** 173 (1931) and Schulz, Becker: *Ibid.,* **203,** 157 (1931).

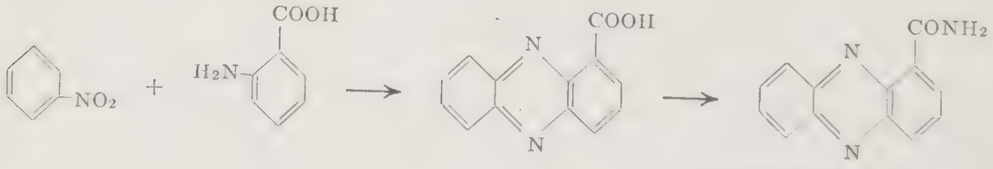
[189] Literature: Schultz: "Farbstofftabellen," 7th ed., Vol. **I,** No. 1873, p. 633; Winterstein-Trier: "Alkaloids," 2nd ed., Berlin, Gebr. Bornträger, 1931, p. 575; Isolation: Ullmann: "Encyclopaedie der technischen Chemie," 2nd ed., Vol. **II,** p. 290; Occurrence: Rupe: "Naturfarbstoffe," Vol. **I,** p. 240.

are derived from the ammonium form. The constitution of berberine has been ascertained by degradation and confirmed by synthesis[190,191]:



Derivatives of Pyrazine

Chlororaphin,[192] $C_{26}H_{20}O_2N_6$ [green crystals, m.p, 229-230° (under nitrogen)] is a pigment produced by *Bacillus chlororaphis G* and *S* when cultivated on a nutrient medium which contains, in addition to inorganic salts, only glycerol and asparagine. Chlororaphin oxidizes in the air to oxychlororaphin (pale-yellow needles, m.p. 241°), with the formula $C_{13}H_9ON_3$. Oxychlororafin is the amide of a phenazine carboxylic acid, as on boiling with potassium hydroxide one molecule of ammonia is liberated with the formation of an acid, $C_{13}H_8O_2N_2$, which is decarboxylated to phenazine on being distilled with soda-lime. It has been identified as the amide of phenazine-α-carboxylic acid by synthesis from nitrobenzene and anthranilic acid:


Oxychlororafin

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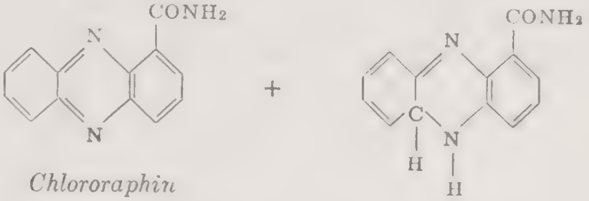
[190] W. H. Perkin, Jr., Rây, Robinson: *J. Chem. Soc.*, **127**, 740 (1925); cf. also Pictet, Gams: *Ber.*, **44**, 2480 (1911) and Haworth, W. H. Perkin, Jr., Rankin: *J. Chem. Soc.*, **125**, 1686 (1924).

[191] Späth, Quientsky: *Ber.*, **58**, 2267 (1925).

[192] Kögl, Postowsky: *Ann.*, **480**, 280 (1930); the work of Lasseur and Girardet is surveyed here; Kögl, Tönnis [*Ann.*, **497**, 265 (1932)] discuss the quinhydrone formula in relation to a possible monoradical structure; Lasseur: *Chem. Zentr.*, **1938**, I, 342; Lasseur, Vernier: *Ibid.*, **1938**, I, 343.

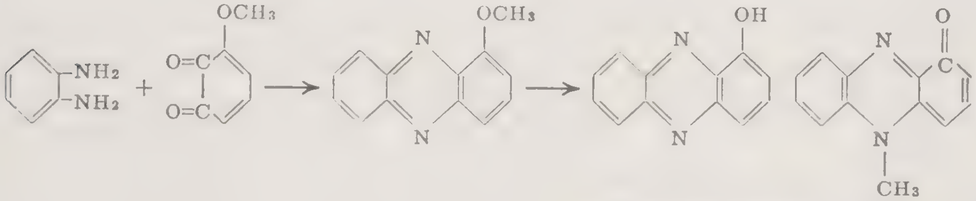
Reduction of oxychlororaphin converted it into a compound of quinhydrone character identical with chlororaphin:



Chlororaphin

Xanthoraphin, which appears in the literature, is identical with oxychlororaphin.

Pyocyanine[193] (I), $C_{26}H_{20}O_2N_4$ (blue needles, m.p. 133°), is a product of *Bacillus pyocyaneus* which is a frequent parasite on the human skin, responsible for the bluish color of perspiration and the color of pus, etc. It is converted by alkali into a yellow compound, hemipyocyanine, $C_{12}H_8ON_2$, which has been identified with α-hydroxyphenazine synthesized in the following manner:



α-Hydroxyphenazine (I)

Pyocyanine is obtained by heating α-hydroxyphenazine with dimethyl sulfate and treating with alkali. A bimolecular formula[194] was originally assumed for pyocyanine, but it was established that the compound exists in aqueous solution as a semiquinone; determination of the molecular weight (in acetic acid) naturally gave values demanded by the bimolecular formula.[195] Finally, however, the radical[196] was isolated as a perchlorate, the molecular weight and paramagnetic nature of which confirmed the monomolecular state of pyocyanine.

For the production[197] of pyocyanine the bacillus is cultivated on "Ragitbouillon Merck" at pH 7.8-8.0 or better on peptone-sodium chloride-gelatin nutrient medium.

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[193] Wrede, Strack: *Z. physiol. Chem.*, **140**, 1 (1924); **142**, 103 (1925); **177**, 177 (1928); **181**, 58 (1929); *Ber.*, **62**, 2051 (1929).

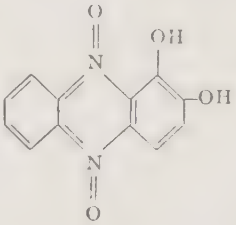
[194] Friedheim, Michaelis: *J. Biol. Chem.*, **91**, 355 (1931); Elema: *Rec. trav. chim.*, **50**, 807 (1931); Michaelis: *J. Biol. Chem.*, **92**, 211 (1931).

[195] Hagemeier: *Dissertation*, Griefswald, 1933.

[196] Elema, Sanders: *Rec. trav. chim.*, **50**, 796 (1931).

[197] Kuhn, Schön: *Ber.*, **68**, 1537 (1935); cf. Pouchkareva, Posstowskij: *J. gen. chem. (U.S.S.R.)*, **8**, 158 (1938); *Chem. Zentr.*, 1939, I, 4328.

A pigment, $C_{12}H_8O_4N_2$ (purple crystals, m.p. 236°), was obtained from *Chromobacterium iodinum.* Distillation with zinc dust gave phenazine; the action of acetic anhydride resulted in the elimination of two oxygen atoms, and catalytic hydrogenation caused the replacement of two oxygen atoms by two hydrogen atoms. The di-N-oxide of 1-hydroxyphenazine behaved very similarly, and as the absorption spectrum of the new coloring matter resembled that of alizarin rather than that of quinizarin, it was formulated as 1,2-dihydroxyphenazine-di-N-oxide[198]:



Lyochromes[199]

The group name *lyochrome*[200] connotes a number of water-soluble nitrogenous pigments with a yellow color and green fluorescence, individual members of which are termed flavins.[201] The identity of one flavin, lactoflavin, with vitamin B_2 is finally established, but it is still not certain that lyochromes other than lactoflavin exist, as the isolated products resemble lactoflavin very closely. Blyth[202] was the first to extract in an impure state the yellow-green coloring matter of milk. Later Bleyer and Kallmann[203] described a nitrogenous pigment, lactochrome, from milk, and Banga and Szent-György[204] obtained a golden-yellow respiratory co-ferment preparation of an extract of pig's heart which they termed *cytoflav.* Finally, Warburg and Christian[205] announced the isolation of a yellow oxidation ferment from yeast.

The yellow ferment, molecular weight 80,000, consists of a colloidal carrier and an active group, a yellow pigment, which can be dissociated from the carrier by the action of methanol. This active group, on irradiation in alkaline solution, passes into a derivative,[206] $C_{13}H_{12}O_2N_4$, which on warming with baryta was broken down into urea and a compound,[207] $C_9H_{10}O_2N_2$. The yellow ferment is an oxidation ferment; but it functions as a carrier of hydrogen and is thus active in anaerobic respiration, and is unaffected by carbon monoxide or hydrocyanic acid. This was as far as the chemical investigation was prosecuted by Warburg and Christian. Meanwhile Ellinger and Koschara and also Kuhn, György and Wagner-Jauregg had isolated the same yellow pigment as was obtained from the oxidation ferment, and with their almost simultaneous preparation of crystalline products[208] research was intensified, and the constitution of lactoflavin was quickly completely elucidated.

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[198] Clemo, McIlwain: *J. Chem. Soc.*, 1938, 479.

[199] Reviews: Kuhn: *Angew. Chem.*, 49, 6 (1936); Weygand: *Chemiker Ztg.*, **61**, 545 (1937). Rudy: *Fortschr. Chem. org. Naturstoffe*, **2**, 61 (1939).

[200] Comparison of lyochromes and lypochromes, see under lypochromes, Ellinger, Koschara: *Ber.*, **66**, 315 (1933).

[201] Kuhn, György, Wagner-Jauregg: *Ber.*, **66**, 317 (1933); cf. Warburg, Christian [*Biochem. Z.*, **266**, 377 (1933)]: who do not favor the above nomenclature.

[202] Blyth: *J. Chem. Soc.*, **35**, 530 (1879).

[203] Bleyer, Kallmann: *Biochem. Z.*, **155**, 54 (1925).

[204] Banga, Szent-György: *Biochem. Z.*, **246**, 203 (1932).

[205] Warburg, Christian: *Naturwiss.*, **20**, 688, 980 (1932); *Biochem. Z.*, **254**, 438 (1932); **257**, 492 (1933); **258**, 496 (1933); **260**, 499 (1933); **263**, 228 (1933); **266**, 377 (1933) (review) purification: Theorell: *Naturwiss.*, **22**, 289 (1934); *Biochem. Z.*, **272**, 155 (1934); cf. also French P. 765,687 (Schering-Kahlbaum); British P. 495,718 (I. G.), *Chem. Zentr.*, 1939, I, 4090.

[206] This observation agrees with the subsequent statements of Kuhn, György and Wagner-Jauregg on the degradation of lactoflavin by alkaline photolysis (see below).

The lyochromes are very widely distributed in the plant and animal kingdoms as may be appreciated, remembering the identity of lactoflavin and vitamin B_2, from the earlier statements[209] of the vitamin B_2 content of various materials. It seems that, although lyochromes can be synthesized by yeasts and bacteria,[210] animals are unable to synthesize their own vitamin B_2 requirements and that flavins found in higher animals are products of vegetable nutrition. Lactoflavin[211] is a constant constituent of green leaves and finds its way into milk in the hay used as fodder. Flavins occur partly in the free form[212] and to some extent as in the yellow oxidation ferment of Warburg where the flavin is bound to protein[213]; similarly liver contains a flavoprotein[214] which is possibly identical with the yellow ferment.

The lyochromes are usually separated by precipitation procedures[215] and most satisfactorily by purification through salts with heavy metals or by adsorption processes[216]; more recently, in spite of their solubility in water, chromatographic analysis has also found some application[217] here. The flavins may be easily detected by the fluorescence[218] of their solutions.

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[207] Kuhn, Rudy [*Ber.*, **67**, 892 (1934) especially p. 895] were unable to obtain the compound $C_9H_{10}O_2N_2$ of Warburg and Christian. They believe it to be carboxylic acid isolated by Kuhn and his co-workers mixed with a by-product (alloxazin) (see below).

[208] Ellinger, Koschara: *Ber.*, **66**, 315 808, 1411 (1933); Kuhn, György, Wagner-Jauregg: Ber., **66**, 317, 576, 1034 (1933); Kuhn, Wagner-Jauregg: *Ber.*, **66**, 1577 (1933); Kuhn, Rudy, Wagner-Jauregg: *Ber.*, **66**, 1950 (1933); cf. also György, Kuhn, Wagner-Jauregg: *Naturwiss.*, **21**, 560 (1933); *Klin. Wochenschrift.*, **12**, 1241 (1933); claim to priority: Warburg, Christian: *Ber.*, **69**, 228 (1935).

[209] Aykroyd, Roscoe: *Biochem. J.*, **23**, 483 (1929); Aykroyd: *Ibid.*, **24**, 1479 (1930); Chick, Copping: *Ibid.*, **24**, 1764 (1930); Roscoe: *Ibid.*, **24**, 1754 (1930); **25**, 1205, 2050 (1931); György, Kuhn, Wagner-Jauregg: *Z. physiol. Chem.*, **223**, 21, 27, 241 (1934); György, v. Klaveren, Kuhn, Wagner-Jauregg: *Ibid.*, **223**, 236 (1934).

[210] Warburg, Christian: *Biochem. Z.*, **266**, 377 (1933); van Veen, Mertens: *Rec. trav. chim.*, **53**, 257, 398 (1934).

[211] Kuhn, Kaltschmitt: *Ber.*, **68**, 128 (1935); detection in other materials: Kuhn, Grundmann: *Ber.*, **67**, 341 (1934); Karrer, Schöpp: *Helv. Chim. Acta*, **17**, 771, 1013 (1934); Stern: *Nature*, **133**, 178 (1934); Warburg, Christian: *Biochem. Z.*, **266**, 377 (1933); Kaltschmitt, see Wagner-Jauregg: *Angew. Chem.*, **47**, 318 (1934); Kuhn, Wagner-Jauregg, Kaltschmitt: *Ber.*, **67**, 1452 (1934); v. Euler, Adler, Schlötzer: *Z. physiol. Chem.*, **226**, 88 (1934); Wagner-Jauregg, Wollschitt: *Naturwiss.*, **22**, 107 (1934); v. Euler, Adler: *Z. physiol. Chem.*, **223**, 105 (1934); Willstaedt: *Chem. Zentr.*, **1935**, I, 2542; *Svensk. Kem. Tid.*, **46**, 259 (1934); Karrer, v. Euler, Schöpp: *Arkiv. Kem. Mineral. Geol. (B)*, **11**, No. 54 (1935). Urochrome, which is responsible for the normal color of urine, probably does not belong to the flavin group.

[212] v. Euler, Adler: *Z. physiol. Chem.*, **228**, 1 (1934).

[213] Theorell: *Naturwiss.*, **22**, 289, 290 (1934).

[214] György, Kuhn, Wagner-Jauregg: *Z. physiol. Chem.*, **223**, 241 (1934).

[215] Guha: *Biochem. J.*, **25**, 945 (1931); György, Kuhn, Wagner-Jauregg: *Z. physiol. Chem.*, **223**, 21, 27, 241 (1934); György, v. Klaveren, Kuhn, Wagner-Jauregg: *Ibid.*, **223**, 236 (1934).

[216] Bleyer, Kallmann: *Biochem. Z.*, **155**, 54 (1925); Warburg, Christian: *Ibid.*, **258**, 496 (1933); **263**, 228 (1933); **266**, 377 (1933); Wagner-Jauregg, Möller, Rauen: *Z. physiol. Chem.*, **231**, 59 (1935); Theorell: *Biochem. Z.*, **278**, 263 (1935); Weygand, Stocker: *Z. physiol. Chem.*, **247**, 167 (1937).

Lactoflavin. Ellinger and Koschara[219] isolated three pigments, lactoflavin *a, b,* and *c,* from the whey of cow's milk. They are distinguished by analyses and crystalline form but behave in a manner recalling that first observed by Warburg and Christian[220] in a preparation of the yellow oxidation ferment; on irradiation they are converted into pigments which are soluble in chloroform.

Kuhn, György and Wagner-Jauregg[221] had also isolated a coloring matter from whey which showed an extraordinary resemblance to ovoflavin (q.v.) previously isolated by them from egg-white. The low tinctorial power[222] of Ellinger and Koschara's first pigments is striking, but a lactoflavin *d* of these workers appears to be identical with the lactoflavin of Kuhn and his co-workers; indeed it has been suggested by Kuhn that the difference between the preparations of the two schools is attributable to the fact that, whereas Ellinger and Koschara effected adsorption on fuller's earth at the neutral point, Kuhn and his collaborators adsorbed from an acid solution.

Pure lactoflavin[223] crystallizes in stellate needles (m.p. 293°) which are optically active in alkaline solution, $[\alpha]$ in $20N$ NaOH $= -100°$, and have the formula $C_{17}H_{20}O_6N_4$ (absorption bands, 445-372-269-225 $m\mu$). The neutral solution shows an intense fluorescence[224] which disappears on addition of acid or alkali. 5400 liters of whey yielded almost 1 gram of pigment.

Lactoflavin is stable toward acids, oxidizing agents, bromine, and nitrous acid, but is destroyed by hot alkali. Reducing agents (sodium hydrosulfite, zinc dust, catalytic methods) convert it into leuco-lactoflavin, which regenerates lactoflavin on shaking with air. *E*, the redox potential at pH $7.0 = -0.185$ volt,[225] so that the pigment is a weak oxidizing agent and the leuco-compound a strong reducing agent. The action of zinc, tin or sodium amalgam in mineral acid solution gives rise to a red intermediate which is a monohydrocompound with the characteristics of a free radical.[226]

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[217] Weygand, Stocker: *Z. physiol. Chem.,* **247,** 167 (1937).

[218] For example: Karrer, Fritzsche: *Helv. Chim. Acta,* **18,** 911 (1935); Supplee, Ansbacher, Flannigan, Hanford: *J. Dairy Sci.,* **19,** 215 (1936); Weisberg, Levin: *Ind. Eng. Chem. (Anal. Ed.),* **9,** 523 (1937).

[219] Ellinger, Koschara: *Ber.,* **66,** 808 (1933); illustrations of crystals of lactoflavin: Ellinger, Koschara: *Ber.,* **66,** 808 (1933); analyses and properties: Ellinger, Koschara: *Ber.,* **66,** 1411 (1933).

[220] Warburg, Christian: *Naturwiss.,* **20,** 980 (1932).

[221] Kuhn, György, Wagner-Jauregg: *Ber.,* **66,** 1034 (1933).

[222] Kuhn, Wagner-Jauregg: *Ber.,* **66,** 1577 (1933); cf. also Koschara: *Ber.,* **67,** 761 (1934), especially p. 763 and note 2. Determination of lactoflavin in natural products: Lunde, Kringstad, Olsen: *Z. physiol. Chem.,* **260,** 141 (1939).

[223] Preparation and purification: cf. Kuhn, Rudy, Wagner-Jauregg: *Ber.,* **66,** 1950 (1933); D. R. P. 638,138 (Schering-Kahlbaum), *Chem. Zentr.,* **1937,** I, 1732, and 638,822 (Schering-Kahlbaum), *Chem. Zentr.,* **1937,** I, 4395; U. S. P. 2,139,857 (S.M.A. Corp.), *Chem Zentr.,* **1939,** I, 3930.

[224] Kuhn, Rudy: *Ber.,* **68,** 169 (1935); Kuhn, Rudy, Weygand: *Ber.,* **68,** 625 (1935); Karrer, Fritzsche: *Helv. Chim. Acta,* **18,** 1026 (1935).

[225] Kuhn, Wagner-Jauregg: *Ber.,* **67,** 361 (1934); Kuhn, Moruzzi: *Ber.,* **67,** 1220 (1934); cf. Stern: *Nature,* 132, 784 (1933); 133, 178 (1934); Bierich, Lang, Rosenbohm: *Naturwiss.,* **21,** 496 (1933); Bierich, Lang: *Z. physiol. Chem.,* **223,** 180 (1934); cf. Stern: *Ber.,* **67,** 654 (1934).

Two properties have proved valuable in the determination of the constitution of lactoflavin, *i.e.*, its sensitiveness toward light and its instability toward alkali; three regions of the molecule were thus disclosed. One part with two atoms of nitrogen is that which is easily destroyed by alkali; the second which is richer in oxygen is split off by irradiation; the third, more stable residue still contains two weakly basic nitrogen atoms. The second part of the molecule contains four hydroxyl groups, as a tetraacetyl compound was obtained; moreover, removal of this portion by photolysis left a compound, lumilactoflavin, $C_{13}H_{12}O_2N_4$, which could no longer be acetylated. If lactoflavin was heated with alkali, urea was split off and a pale-yellow pigment was obtained which was insoluble in chloroform. It takes up four acetyl groups. Now oxidation of lactoflavin [227] with lead tetraacetate gave 0.775 mol of formaldehyde, whereas similar treatment of lumilactoflavin gave no volatile aldehyde. It follows that lactoflavin contains a primary hydroxyl group adjacent to another hydroxyl group, and the highly oxygenated part of the molecule therefore possesses the constitution —CH(OH)—CH(OH)—CH(OH)—CH_2OH. Alkaline photolysis may thus be represented as the replacement of the side-chain $C_4H_9O_4$ by hydrogen:

$$C_{17}H_{20}O_6N_4 - C_4H_8O_4 \longrightarrow C_{13}H_{12}O_2N_4$$

Lumilactoflavin has, as shown above, the formula $C_{13}H_{12}O_2N_4$, and forms prisms (m.p. 320-321 °); it is optically inactive. One active hydrogen [228] atom is revealed by the Zerewitinoff determination. On warming with baryta water urea [229] is split off, and a number of yellow fission products are obtained by treatment with sodium hydroxide, of which the chief has the composition $C_{12}H_{12}O_3N_2$. It loses carbon dioxide on being sublimed, passing into a compound $C_{11}H_{12}ON_2$ (yellow prisms, m.p. 169-170°), which is therefore formed from lumilactoflavin according to the equation:

$$C_{13}H_{12}O_2N_4 + 2H_2O = CO(NH_2)_2 + CO_2 + C_{11}H_{12}ON_2$$

Lumilactoflavin, however, contains one alkylamide [230] group and was eventually shown by degradation to have the following constitution (I):

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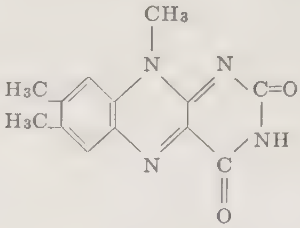
[226] Kuhn, Wagner-Jauregg: *Ber.*, 67, 361 (1934); Kuhn, Moruzzi: *Ber.*, 67, 888 (1934); Holiday, Stern: *Ber.*, 67, 1352 (1934).

[227] Reduction products of lactoflavin, see Ellinger, Koschara: *Ber.*, 66, 1411 (1933); Kuhn, Ströbele: *Ber.*, 70, 753 (1937).

[228] Kuhn, Rudy: *Ber.*, 67, 892, 1298 (1934); cf. Stern, Holiday: *Ber.*, 67, 1104 (1934).

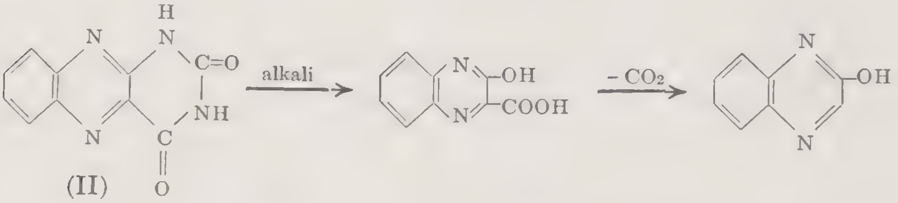
[229] Warburg, Christian: *Biochem. Z.*, 258, 496 (1933); 263, 228 (1933).

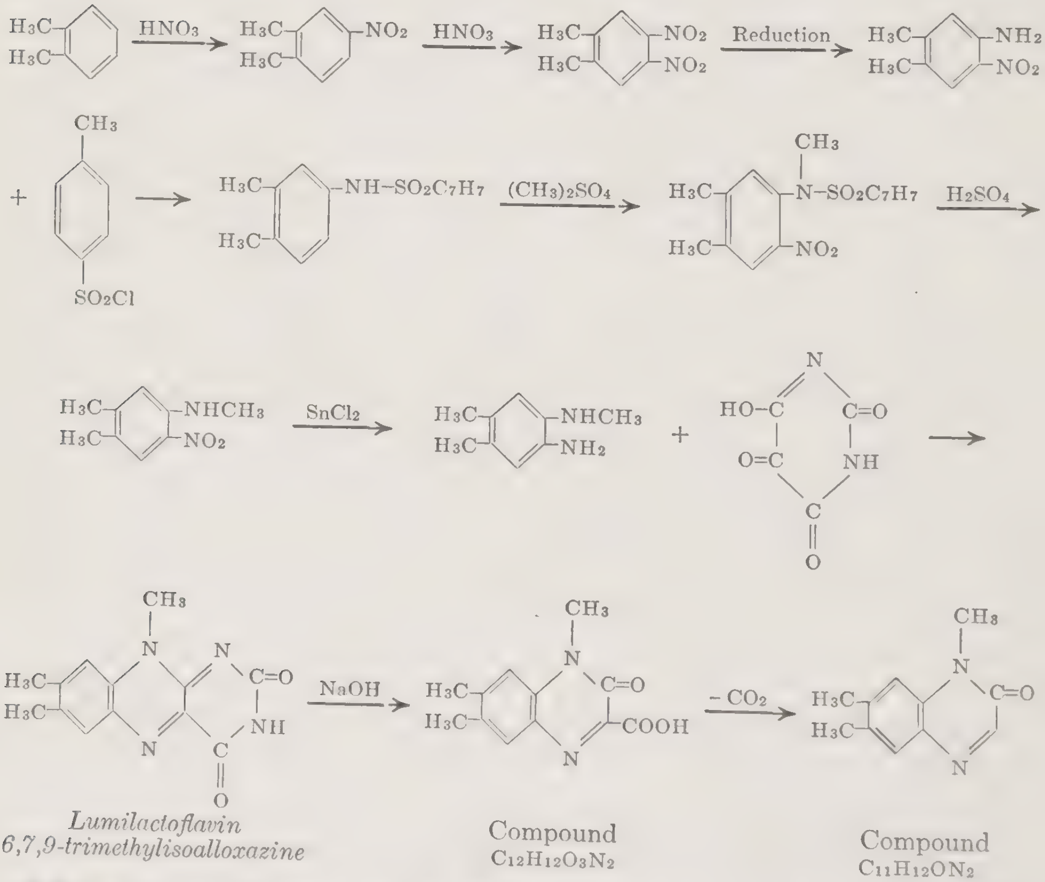
[230] Kuhn, Rudy: *Ber.*, 67, 1298 (1934); Determination of alkylimide: Kuhn, Roth: *Ber.*, 67, 1458 (1934).



(I)

The removal of urea is thus directly comparable with the fission of alloxazine (II) to 2-hydroxyquinoxaline-3-carboxylic acid. The synthesis [231] and degradation [231] are indicated in the following scheme:

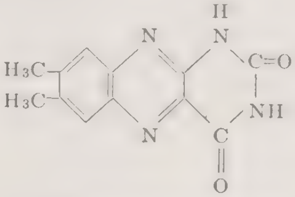

(II)
alkali
- CO2


HNO3
HNO3
Reduction
(CH3)2SO4
H2SO4
SnCl2
NaOH
- CO2
Lumilactoflavin
6,7,9-trimethylisoalloxazine
Compound
C12H12O3N2
Compound
C11H12ON2

---

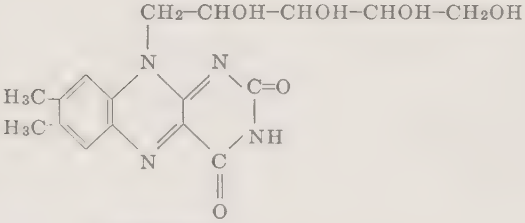
[231] Kuhn, Reinemund, Weygand: *Ber.*, 67, 1460 (1934); Preliminary work: Kuhn, Weygand: *Ber.*, 67, 1409, 1459 (1934); cf. Stern, Holiday: *Ber.*, 67, 1442 (1934); Kuhn, Rudy: *Ber.*, 68, 300 (1935); Kuhn, Rudy, Reinemund: *Ber.*, 68, 170 (1935).

Irradiation of neutral [232] or weakly acid flavin solutions in daylight or sunlight and in the presence of air yielded another photolysis product, lumichrome,[233] $C_{12}H_{10}O_2$ (straw-yellow crystals melting above 300°), which is free from methoxyl and methylimide groups and is in fact identical with 6,7-dimethylalloxazine:



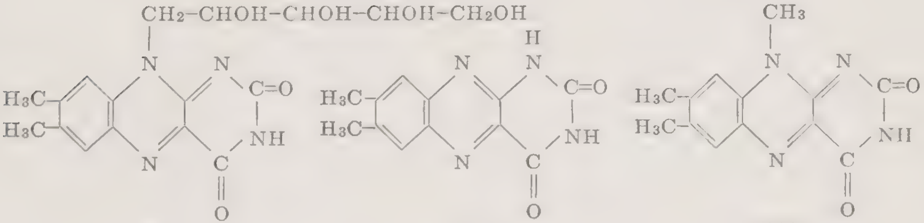
6,7-Dimethylalloxazine

Lactoflavin is, therefore, from consideration of the above facts, given the constitution [234]:



Lactoflavin

It was further shown that photochemical decomposition [235] of (I)



(I) (II) (III)

afforded the alloxazine (II) in neutral solution and the alloxazine and 9-methylisoalloxazine (III) in alkaline solution, thus confirming the structure shown above.

The parent substance [236] quinoxaline, with two atoms of nitrogen, may be reduced in the same manner as its higher derivatives, and in this respect the coupling[237] with the alkali-labile ring system and further addition of a polyhydroxylated component is almost without effect. The grouping N—CO—NH—CO in the additional ring system is responsible for the deepening in color. Without the sugar-like side-chain no vitamin characteristics are observed, and it is mainly the association with protein which imparts ferment character to the pigment (the natural component contains also phosphoric acid).

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[232] Kuhn, Rudy, Wagner-Jauregg: *Ber.*, **66**, 1950 (1933); Karrer, Köbner, Salomon, Zehender: *Helv. Chim. Acta*, **18**, 266 (1935); Karrer, Musante: *Ibid.*, **18**, 1134 (1935).

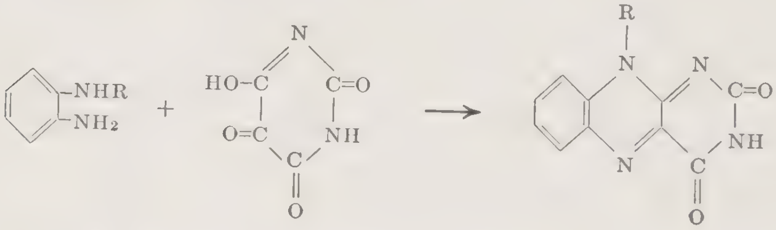
[233] Karrer, Salomon, Schöpp, Schlittler, Fritzsche: *Ibid.*, **17**, 1010 (1934); Kuhn, Rudy: *Ber.*, **67**, 1936 (1934).

[234] Karrer, Salomon, Schöpp, Schlittler: *Helv. Chim. Acta*, **17**, 1165 (1934); Kuhn, Wagner-Jauregg: *Ber.*, **67**, 1770 (1934); Kuhn, Rudy: *Ber.*, **67**, 1826 (1934); claim to priority: Karrer: *Ber.*, **67**, 2061 (1934); Kuhn: *Ber.*, **68**, 172 (1935); further consideration on the photolysis: Karrer, Köbner, Salomon, Zehender: *Helv. Chim. Acta*, **18**, 266 (1935); Karrer, Meerwein: *Ibid.*, **18**, 480, 1126 (1935).

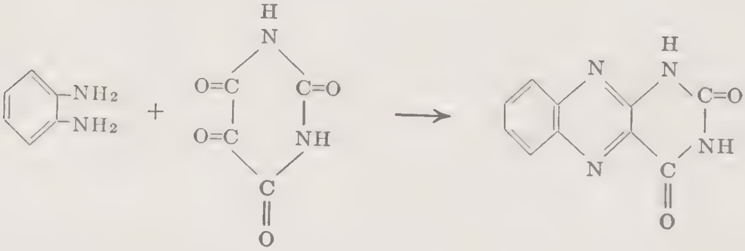
[235] Karrer, Salomon, Schöpp, Schlittler: *Helv. Chim. Acta*, **17**, 1165 (1934); Kuhn, Wagner-Jauregg: *Ber.*, **67**, 1770 (1934); Kuhn, Rudy: *Ber.*, **67**, 1826 (1934).

[236] Cf. the earlier work of Kuhn, Bär: *Ber.*, **67**, 898 (1934), where the photochemical decomposition of 2-tetrahydroxybutyl-quinoxaline is described; for the constitution, cf. Kuhn, Rudy: *Ber.*, **67**, 1125 (1934).

Synthesis has revealed that natural lactoflavin is that stereoisomeride of the constitution given above in which the sugar residue has the configurational relationship of *d*-ribose. Syntheses of lactoflavin and similar compounds differ only in details and not in principle, as the following summary shows. All are based on the condensation of a N-monosubstituted aromatic *o*-diamine with alloxan in weakly acid solution:



which is itself an extension of the well-known condensation of aromatic *o*-diamines with alloxan to give alloxazines:



Summary of syntheses[238]:

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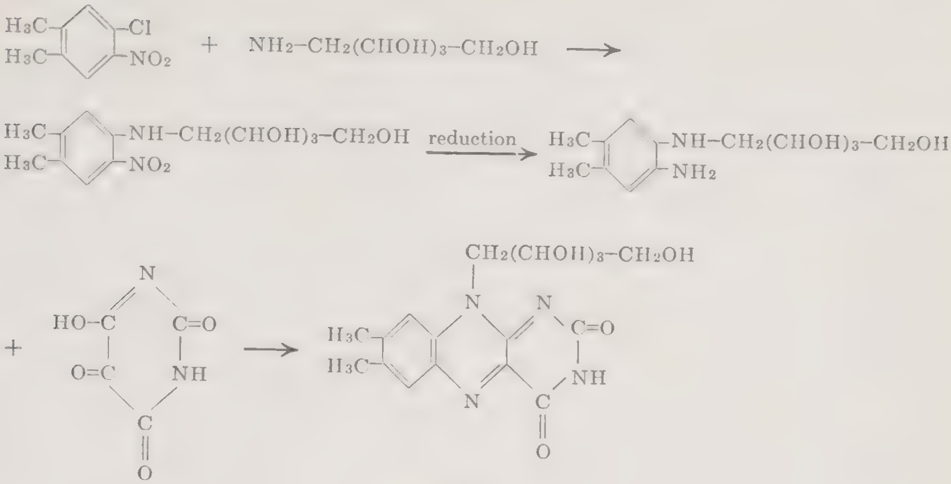
[237] Kuhn, Moruzzi: *Ber.*, **67**, 1220 (1934); Wagner-Jàuregg, Rauen, Möller: *Z. physiol. Chem.*, **228**, 273 (1934).

[238] Karrer, Salomon, Schöpp, Schlittler: *Helv. Chim. Acta*, **17**, 1165 (1934); Karrer, Schlittler, Pfaehler, Benz: *Ibid.*, **17**, 1516 (1934); Kuhn, Weygand: *Ber.*, **67**, 1939, 1941, 2084 (1934); Karrer, Schöpp, Benz, Pfaehler: *Helv. Chim. Acta*, **18**, 69 (1935); Kuhn, Weygand: *Ber.*, **68**, 166, 1282, 1001 (1935); Kuhn: *Nature*, 135, 185 (1935); synthesis of lactoflavin: Karrer, Schöpp, Benz: *Helv. Chim. Acta*, **18**, 426 (1935), and v. Euler, Karrer, Malmberg, Schöpp, Benz, Becker, Frei: *Ibid.*, **18**, 522 (1935); Kuhn, Reinemund, Kaltschmitt, Ströbele, Trischmann: *Naturwiss.*, **23**, 260 (1935); Kuhn, Reinemund, Weygand, Ströbele: *Ber.*, **68**, 1765 (1935); Karrer, Meerwein: *Helv. Chim. Acta*, **18**, 1130 (1935); Br. P. 457,178 (Hoffmann-La Roche), *Chem. Zentr.*, **1937**, I, 2406; Karrer, Salomon, Schöpp, Benz: *Helv. Chim. Acta*, **18**, 1143 (1935); Karrer, Becker, Benz, Frei, Salomon, Schöpp: *Ibid.*, **18**, 1435 (1935); Karrer, Strong: *Ibid.*, **19**, 483 (1936); Karrer, Meerwein: *Ibid.*, **19**, 1190 (1936); Karrer, Salomon, Schöpp, Benz, Becker: *Ibid.*, **18**, 908 (1935); Karrer, Strong: *Ibid.*, **18**, 1343 (1935); Karrer, Naef: *Ibid.*, **19**, 1029 (1936); Karrer, Quibell: *Ibid.*, **19**, 1034 (1936); Kuhn, Ströbele: *Ber.*, **70**, 747 (1937); Kuhn, Cook: *Ber.*, **70**, 761 (1937); Kuhn, Weygand: *Ber.*, **70**, 769 (1937); Karrer: *Ber.*, **70**, 2565 (1937); Kuhn, Desnuelle, Weygand: *Ber.*, **70**, 1293 (1937); Kuhn, Vetter, Rzeppa: *Ber.*, **70**, 1302 (1937); Kuhn, Rudy, Weygand: *Ber.*, **68**, 625 (1935). D.R.P. 679,001, Addn. to D.R.P. 642,148.

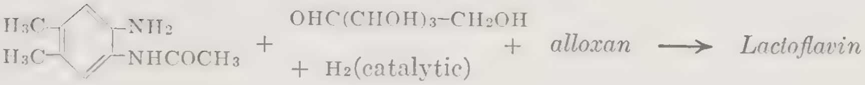
I.



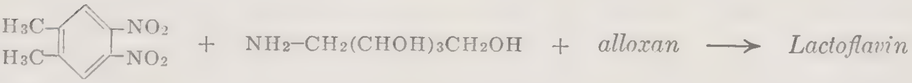
Alloxan

(I) *Lactoflavin*

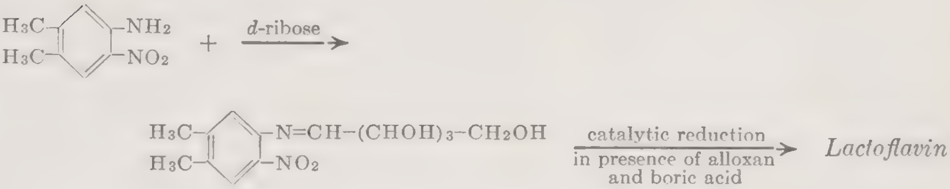
II.



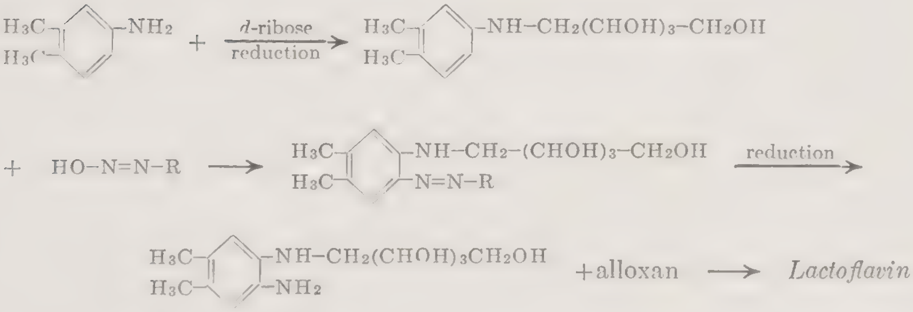
III.



IV.



V.



To summarize therefore, lactoflavin is 6,7-dimethyl-9-(*d*-ribityl)-isoalloxazine.

Lactoflavin provides a further example of that small but growing list of compounds the biological activity of which is not as specific as was at first supposed. In the present instance the synthesis of analogous compounds and their examination [239] for biological activity has revealed that a number of structurally related compounds are also active if the following conditions are fulfilled: The imide group must be free; the two methyl groups attached to the benzene nucleus cannot be dispensed with, although compounds with one methyl group are also somewhat active; the sugar residue cannot be replaced by alkyl, aralkyl or aryl groups, nor is a glycoside linking permissible. Lactoflavin possesses no toxic properties.[240]

Lactoflavin occurs in milk [241] in the free state, but closer examination of the yellow ferment [242] has revealed that here the pigment is bound to phosphoric acid. This lactoflavin phosphoric acid [243] has the formula $C_{17}H_{21}O_9N_4P$ and, it is claimed, has been shown by synthesis to have the constitution:

$$\text{Flavin}\text{—}CH(CH\,.\,OH)_3\text{—}CH_2O\text{—}PO(OH)_2$$ [244]

Karrer [245] on the other hand has expressed doubt of the validity of this conclusion. Cytoflav[246] from heart-muscle is identical[247] with lactoflavin. It is not inappropriate to mention that Theorell [248] has also purified the colloidal carrier of the yellow ferment. Synthetic lactoflavin phosphoric acid may be recombined with the carrier to regenerate a "synthetic" ferment [249]; esterification [250] with phosphoric acid is not essential for the appearance of vitamin activity, but is important in stabilizing the pigment-carrier association. Kuhn [251] has suggested a diagrammatic structural formula in which protein is linked to the phosphoric acid and imide-groups as a salt, an arrangement which would explain the importance of the NH group:

[239] Kuhn, Boulanger: *Ber.*, **69**, 1557 (1935); Kuhn, Rudy: *Ber.*, **69**, 2557 (1935).

[240] Kuhn, Boulanger: *Z. physiol. Chem.*, **241**, 233 (1936); Kuhn: *Klin. Wochenschrift*, **17**, 222 (1938).

[241] Kuhn, Kaltschmitt: *Ber.*, **68**, 386 (1935).

[242] Theorell: *Biochem. Z.*, **275**, 37, 344, 416 (1934); **278**, 263 (1935); **279**, 186 (1935).

[243] Kuhn, Rudy: *Ber.*, **68**, 383 (1935); Kuhn: *Bull. soc. chim. biol.*, **17**, 905 (1935); Kuhn, Rudy: *Z. physiol. Chem.*, **239**, 47 (1936); Br. P. 451,938 (I. G.), *Chem. Zentr.*, **1937**, I, 663.

[244] Kuhn, Rudy, Weygand: *Ber.*, **69**, 1543 (1936).

[245] Karrer, Frei, Meerwein: *Helv. Chim. Acta*, **20**, 79 (1937).

[246] Banga, Szent-Györgi: *Biochem. Z.*, **246**, 203 (1932).

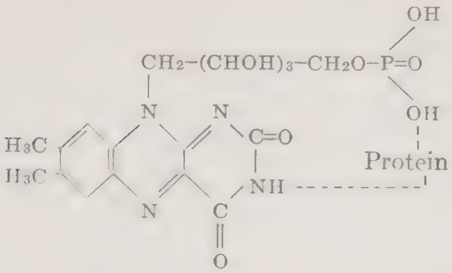
[247] Kuhn, Rudy: *Z. physiol. Chem.*, **239**, 47 (1936).

[248] Theorell: *Biochem. Z.*, **278**, 263 (1935); **290**, 293 (1937).

[249] Kuhn, Rudy: *Ber.*, **69**, 1974, 2557 (1936); a number of important biological findings are embodied in the latter paper; cf. also Wagner-Jauregg, Ruska: *Ber.*, **66**, 1298 (1933); v. Euler, Karrer, Adler: *Ark. Kemi. Mineral Geol. (B)*, **11**, No. 33 (1934); Kuhn, Rudy, Weygand: *Ber.*, **69**, 2034 (1936); also v. Euler, Karrer, Malmberg: *Helv. Chim. Acta.*, **18**, 1336 (1935), and Karrer, Quibell: *Ibid.*, **19**, 1034 (1936); Kuhn, Weygand: *Ber.*, **68**, 1282 (1935); Rudy: *Z. physiol. Chem.*, **242**, 198 (1936); Haas: *Biochem. Z.*, **290**, 291 (1937).

[250] Kuhn, Rudy: *Z. physiol. Chem.*, **239**, 47 (1936).

[251] Cf. also Kuhn, Boulanger: *Ber.*, **69**, 1557 (1936); Rudy: *Naturwiss.*, **24**, 501 (1936); on the nature of the protein: Kuhn, Desnuelle: *Ber.*, **70**, 1907 (1937).



The biological importance of lactoflavin and the lyochromes generally appears to reside in their ability, by assuming the leuco-form, to take up hydrogen from donators and transfer it to acceptors when they themselves become reoxidized. In biological work the important fact has emerged that normal metabolism utilizes a series of such reducing agents such as ascorbic acid, glutathione, and lactoflavin, graded and of progressively more negative reduction potential, so that hydrogen of an oxidizable substrate passes from one to the other until it is finally united to atmospheric oxygen.

Ovoflavin. This flavin,[252] isolated from egg-white, forms orange needles (m.p. 265°) with the formula $C_{17}H_{20}O_6N_4$, and has the same spectrum as lactoflavin. Its tetraacetate melts, like that of lactoflavin, at 242° and no depression was observed on mixing the two. Moreover, lumichrome is obtained by irradiation of ovoflavin as well as lactoflavin.[253] Nevertheless, in spite of these similarities, there may be differences in the sugar residues, especially as the biological identity is not free from doubt.[254] Ovoflavin is prepared from freshly collected egg-white or from commercial dried egg albumen by extracting with methanol, adsorbing the pigment on fuller's earth, and then eluting it with dilute pyridine. Thirty kg. of egg albumen, equivalent to 10,000 eggs, yielded 30 mg. of ovoflavin after three crystallizations, although Karrer [255] isolated an ovoflavin (long needles, m.p. 284°) in a yield of 15 mg from 1000 fresh eggs.

Flavin from liver (formerly termed hepaflavin). Flavin from liver,[256] *e.g.*, horse-liver, has the formula $C_{17}H_{20}O_6N_4$ (brown needles, m.p. 280°), and absorption bands [257] at 442-365-263 mμ have been observed. The alkaline fission test (color reaction) and optical activity as well as the formation of lumilactoflavin have led Kuhn and Wagner-Jauregg [258] to pronounce the flavins of milk, yeast and liver to be identical. Lactoflavin has also been isolated from liver as its phosphoric ester.[259]

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[252] Kuhn, György, Wagner-Jauregg: *Ber.*, **66**, 317, 576 (1933).

[253] Karrer, Schöpp: *Helv. Chim. Acta*, **17**, 1557 (1934).

[254] Kuhn: *Angew. Chem.*, **47**, 105 (1934); v. Euler, Karrer, Adler: *Ark. Kemi. Mineral. Geol. (B)*, **11**, No. 33 (1935); v. Euler, Karrer, Adler, Malmberg: *Helv. Chim. Acta*, **17**, 1157 (1934).

[255] Karrer, Schöpp: *Helv. Chim. Acta*, **17**, 735 (1934).

[256] Schöpp, Karrer, Salomon: *Ibid.*, **17**, 419 (1934); cf. also Stern: *Ber.*, **66**, 555 (1933).

[257] Stern: *Z. physiol. Chem.*, **212**, 207 (1932); *Nature*, **132**, 784 (1933); Stern postulated its identity with the photolytic product of Warburg's yellow oxidation ferment from the equivalence of their absorption bands; cf. also Bierich, Lang, Rosenbohm: *Naturwiss.*, **21**, 496 (1933).

[258] Kuhn, Wagner-Jauregg: *Ber.*, **67**, 1772 (1934); Stern: *Biochem. J.*, **28**, 949 (1935).

Lyochromes occurring in urine. As the lyochromes are soluble in water it was to be expected that they would be excreted in the urine. In fact, they are not found in faeces, but urine contains at least four lyochromes in a dilution of about 1:3,000,000.

Uroflavin,[260] purified by chromatographic analysis, agrees in its absorption spectrum and optical activity with lactoflavin. Moreover, its acetate has the same melting point as that of lactoflavin, and on "alkaline photolysis" it yields a lumiflavin which in crystalline form, decomposition point, and analysis exhibits no considerable difference from lumilactoflavin. The carbon values, however, of the original pigment show a deficit of 1 per cent, and on that account Koschara believes that lactoflavin and uroflavin are not identical.

Aquoflavin[261] owes its name to the fact that its chromatogram may be developed by water alone. It has not been obtained in the crystalline form and its properties have been investigated using highly purified solutions. The third coloring matter gives photolysis products on irradiation which indicate a lyochrome nature.

Uroerythrin[262] is an orange-red pigment of urine which possesses reducing properties and is decolorized by light. It exhibits absorption bands at 525-540 and 490-500 $m\mu$ and is possibly identical with Thannhauser's xanthorubin.

Flazin[263] is a similar pigment, $C_{18}H_{16}O_5N_2$ (yellow needles, m.p. 218-220°), which has been obtained from Saké-Kasu (Saké grain). It is soluble in alcohol, acetone and acids, with a strong yellow-green fluorescence which disappears on neutralizing the solutions with alkali. Flazin is believed to contain the phenazine ring system.

Renoflavin.[264] A yellow-red hygroscopic mass which possessed a green fluorescence and exhibited vitamin activity was obtained from an extract of ox-kidney. Yellow prismatic needles were finally obtained, but the purification is rendered difficult by the sensitiveness of the compound to light.

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[259] Theorell. Karrer, Schöpp, Frei: *Helv. Chim. Acta,* **18,** 1022 (1935); see also v. Euler, Adler: *Z. physiol. Chem.,* **223,** 105 (1934); *Ark. Kemi, Mineral. Geol. (B),* **11,** No. 28 (1934); fermentation flavin from rice: Yamasaki: *Biochem. Z.,* **300,** 160 (1939).

[260] Koschara: *Ber.,* **67,** 761 1934); Kuhn, Wagner-Jauregg, György: *Ber.,* **66,** 1034 (1933); Kuhn, Wagner-Jauregg: *Ber.,* **66,** 1577 (1933); Koschara: *Z. physiol. Chem.,* **232,** 101 (1935); cf. also Rangier, de Traverse: *Compt. rend.,* **207,** 1257 (1938); **208,** 1345 (1939).

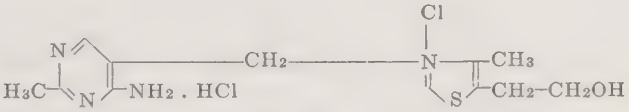
[261] Koschara: *Z. physiol. Chem.,* **229,** 103 (1934); **232,** 101 (1935); cf. also Kuhn, Rudy: *Ber.,* **68,** 300 (1935).

[262] Weiss: *Deut. Arch. klin. Med.,* **177,** 97 (1935).

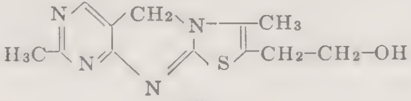
[263] Higashi: *Bull. Inst. Phys. Chem. Research* (Tokyo), **15,** 1060 (1936); *Sci. Papers Inst. Phys. Chem. Research (Tokyo),* **33,** No. 727 (1937).

[264] Guha, Biswas: *Current Sci.,* **2,** 474 (1934); *Chem. Zentr.,* **1934,** *II,* 1327; *Ber.,* **68,** 427 (1935).

Thiochrome, $C_{12}H_{14}ON_4S$ (yellow prisms, m.p. 222°), was isolated from yeast,[265] when it was noticed that its empirical formula differed from that of vitamin B_1 only in containing two less atoms of hydrogen (vitamin B_1, $C_{12}H_{16}ON_4S$). When vitamin B_1 (antineurin or aneurin) was successfully oxidized[266] to thiochrome by potassium ferricyanide in alkaline solution, the close connection between the two was confirmed and the determination of their constitution became interdependent. Aneurin has the constitution[267] (I)

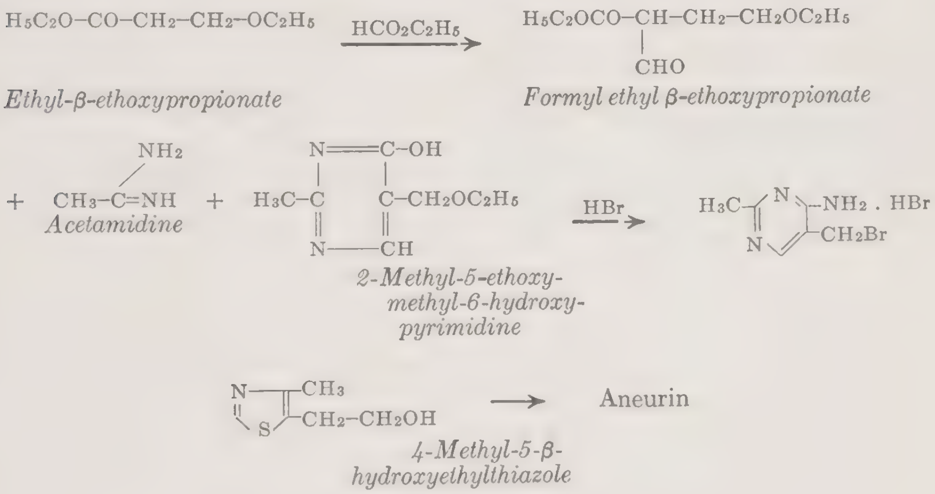


(I)



(II)

and passes by loss of two atoms of hydrogen into thiochrome (II). Of the numerous syntheses of aneurin only the first[268] need be given here:


$H_5C_2O{-}CO{-}CH_2{-}CH_2{-}OC_2H_5$
$HCO_2C_2H_5$
$H_5C_2OCO{-}CH(CHO){-}CH_2{-}CH_2OC_2H_5$
Ethyl-β-ethoxypropionate
Formyl ethyl β-ethoxypropionate
Acetamidine
HBr
2-Methyl-5-ethoxymethyl-6-hydroxypyrimidine
Aneurin
4-Methyl-5-β-hydroxyethylthiazole

Pigments of Unknown Constitution

A pigment[269] has been isolated from a new bacterium. It has the formula $C_{10}H_{12}O_3N$, is soluble in ether and has absorption bands at 546-

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[265] Kuhn, Wagner-Jauregg, van Klaveren, Vetter: *Z. physiol. Chem.*, **234**, 196 (1935).

[266] Barger, Bergel, Todd: *Ber.*, **68**, 2257 (1935); cf. also Swiss Pat. 184,611 (Hoffmann-La Roche), *Chem. Zentr.*, **1937**, I, 1794.

[267] Grewe: *Z. physiol. Chem.*, **242**, 92 (1936); the whole literature is not given here as it is chiefly concerned with aneurin.

[268] Williams, Cline: *J. Am. Chem. Soc.*, **58**, 1504 (1936); Cline, Williams, Finkelstein: *Ibid.*, **59**, 1052 (1937); other syntheses: Todd, Bergel: *J. Chem. Soc.*, **1937**, 364; Andersag, Westphal: *Ber.*, **70**, 2035 (1937); Hoshino, Ohta: *Proc. Imp. Acad. (Tokyo)*, **13**, 101 (1937); *Chem. Zentr.*, **1937**, II, 3894; Imai, Makino: *Z. physiol. Chem.*, **252**, 76 (1938).

[269] Sartory, Meyer, Waeldele: *Bull. Sci. Pharmacol.*, **45**, 302 (1938).

582-620 mμ. The pigment is possibly a quinoneimine of the quinoline series.

Violacein.[270] This pigment is present in *Chromobacterium violaceum* and forms almost black needles (m.p. > 350°) with the probable formula $C_{42}H_{28}O_7N_6$. One of the nitrogen atoms is basic in nature and forms an acetyl compound, but violascein also dissolves in alkalies with a green color. Catalytic hydrogenation results in the absorption of 14-16 atoms of hydrogen.[271] *Pseudomonas fluorescenz* produces a coloring matter [272] with a green fluorescence and the formula $C_{32}H_{41}O_8N_7$. There are few clues to its constitution except that on distillation with lime it yields a compound containing a heterocyclic ring-system. Turfitt [273] has isolated a pigment, bacteriofluorescein, from selected strains of *Bacillus pyocyaneus* grown on asparagene cultures. The compound has the empirical formula $C_4H_7O_2N$ and shows absorption bands at 410 mμ in alkaline and 370 mμ in acid solution.

Urothion, $C_{11}H_{13}O_3N_5S_2$, is a yellow coloring matter of which 40-80 mg. has been isolated from 1000 liters of urine. It forms a tetracetyl derivative, m.p. 220°, contains one amino group, and exhibits the extraordinary behavior of liberating one molecule of methyl mercaptan on catalytic reduction.[274]

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[270] Reilly, Pyne: *Biochem. J.*, **21,** 1059 (1927).

[271] Kögl, Tönnis in Klein: "Handbuch der Pflanzenanalyse," Vol. **III,** 2, p. 1443; Wrede, Kohlhaas: *Z. physiol. Chem.*, **223,** 113 (1934); Tobie: *J. Bact.*, **29,** 223 (1935); Wrede: *Arch. Exp. Path. Pharmakol.*, **186,** 532 (1937).

[272] Turfreijer, Wibaut, Boltjes: *Rec. trav. chim.*, **57,** 1397 (1938).

[273] Turfitt: *Biochem. J.*, **30,** 1323 (1936); **31,** 212 (1937); cf. the pyorubrin of Meader, Robinson, Leonard: *Am. J. Hyg.*, **5,** 682 (1925).

[274] Koschara: *Z. physiol. Chem.*, **263,** 78 (1940).

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